# ECOLOGY, EVOLUTION, AND CONSERVATION OF PLANT–ANIMAL INTERACTIONS ON ISLANDS

**DISSERTATION**

ZUR

**ERLANGUNG DER NATURWISSENSCHAFTLICHEN DOKTORWÜRDE**

(DR. SC. NAT.)

VORGELEGT DER

**MATHEMATISCH-NATURWISSENSCHAFTLICHEN FAKULTÄT**

DER

**UNIVERSITÄT ZÜRICH**

VON

**DENNIS MARINUS HANSEN**

AUS

**DÄNEMARK**

**PROMOTIONSKOMITEE**

**PROF. DR. CHRISTINE B. MÜLLER (VORSITZ)**

**PROF. DR. ELENA CONTI**

**PROF. DR. LUKAS KELLER**

**PROF. DR. H. PETER LINDER**

**ZÜRICH 2006**

# *An* Authentic Narrative

~ of ~

*Travells to* Mauritius

Where Many Years of Fruteful and Pleasaunt Worke

~ Led to ~

* * * * *

a ſtudy Moſt Bizarre of the Mysterious Coloured Nectar

* * * * *

the diſcovery of ſeveral

Pollinationne and Seed Dispersall Interactions new to ſcience, involving

Dragonnes & Geckoes moſt Wonderfulle and Plantes moſt weirde

* * * * *

the resurrection of the olde Geant Tortoise as a Seed Disperser

~ &c. ~

* * * * *

Reported in a Treatise,

Compos'd, Compiled and Methodically Digested

by Dennis Marinus Hansen

~ Traveller, Natural Hiſtorian, and ſcientiſt moſt Eclectic ~

*Being a Diſcourſe not Unworthy the Peruſal of Fellow Schollers,*
*Illuſtrated and Embelliſhed with divers Colour Engravings*

* * * * *

Universitas Turicensis MM,VI

**TO MY PARENTS,**

**WHO LET ME RUN AND GET MUDDY**
**IN THE FORESTS OF MY CHILDHOOD**

"I trust that these, and all other friends who have been in any way interested in my travels and collections, may derive from the perusal of my book, some faint reflexion of the pleasures I myself enjoyed amid the scenes and objects it describes."

– Wallace, 'Malay Archipelago', 1869

# CONTENTS

# GENERAL INTRODUCTION

"Everything that shouldn't be done to an island has been done to Mauritius. Except perhaps nuclear testing."

-Richard Lewis, in 'Last chance to see' by Douglas Adams.

## BACKGROUND

Ever since Darwin's 'Origin' (1859) and Wallace's 'Malay Archipelago' (1869) and 'Island Life' (1880), biologists have been fascinated by islands (e.g. Vitousek *et al.* 1995; Grant 1998), chiefly for two reasons: firstly, because of the often peculiar paths that evolution has taken on islands – which has led to high levels of endemism for many island biotas; secondly, due to the striking simplicity of many island ecosystems in comparison to mainland habitats. Because oceanic islands are isolated by the surrounding sea, not all plant and animal taxa from neighbouring mainland areas have reached them. The oceans have acted as selective barriers and filters, resulting in an asymmetrical subset of mainland taxa that have colonised islands, and a lower overall species diversity. For example, no large non-flying mammals occur naturally on oceanic islands, and neither do some social hymenopterans, such as honey bees. Therefore, island floras and faunas and the resulting ecosystems, differ greatly from comparable mainland areas, and generally comprise much fewer species (Carlquist 1965; MacArthur & Wilson 1967; Carlquist 1974; Whittaker 1998).

Mutualistic plant–animal interactions, such as pollination and seed dispersal, perfectly illustrate these differences in community composition and species interactions between mainland and insular habitats. For example, on islands there are only few species from the major pollinating (e.g. social bees and wasps, and butterflies) and seed dispersing (e.g. large non-flying mammals, and frugivorous birds) mainland groups (Cox *et al.* 1991; Elmqvist *et al.* 1992; Barrett 1996). This has resulted in other animals, which are not normally foraging at flowers and fruits, utilising the otherwise unharvested floral and fruit resources. Island lizards are a good example of this, as many of them have broadened their mainly carnivorous or herbivorous feeding niches to include nectar and fruits in their diets. Indeed, recent reviews have highlighted an emerging pattern of lizards as important mutualistic partners in many insular pollination and seed dispersal interactions (Olesen & Valido 2003; Godínez-Álvarez 2004; Valido & Olesen in press). Similarly, other large reptiles, such as tortoises, are important seed dispersers on some islands (Rick & Bowman 1961; Hnatiuk 1978).

Unfortunately, the traits that cause islands to be of great interest to evolutionary biologists and ecologists – evolution in isolation, and disharmonic and impoverished biotas – are commonly also credited with being the main reason for the fragility of island ecosystems (Simberloff 2000). Wherever humans and their associated pest or domestic animals have arrived on an island, massive extinctions of native and endemic animals

have resulted (e.g. Diamond 1984; Savidge 1987; Olson 1989; Milberg & Tyrberg 1993; Frankham 1998). Similarly, habitat loss and invasive plant species have caused high extinction rates in native and endemic plants on many islands e.g. (Strahm 1994; Bouchet *et al.* 1995; Bernardello *et al.* 2001). Consequently, but much harder to quantify, many mutualistic plant–animal interactions must also have been lost, and for some animal species – and perhaps especially for plant species – this lack of mutualistic partners is likely to be a major post in the extinction debt (*sensu* Tilman *et al.* 1994) incurred in many fragmented insular habitats (Kearns *et al.* 1998; Traveset 1999; Cordeiro & Howe 2001). Furthermore, even if the mutualistic partners are not extinct, the mutualism may be disrupted by invasive species (Traveset & Richardson 2006). There are some studies that have investigated disruptions of mutualisms or loss of mutualistic partners on islands (e.g. Cox & Elmqvist 2000; McConkey & Drake 2002; Hansen *et al.* 2002; Meehan *et al.* 2002; Riera *et al.* 2002; Dupont *et al.* 2004; Traveset & Riera 2005), but more research is needed, especially on how conservation management can take mutualistic interactions into account (Traveset & Richardson 2006; Cheke & Hume in press).

## CONCEPT AND OUTLINE OF THIS THESIS

In my thesis, I attempt to combine the curiosity of a natural historian, the inquisitiveness of an evolutionary ecologist, and the fervour of a conservation biologist. All of my work in this thesis has its deep roots in Mauritius. Mauritius is one of the three Mascarene Islands, and lies approximately 800 km east of Madagascar (Fig. 1). The fate of Mauritius illustrates many of the problems faced by native and endemic biodiversity on isolated oceanic islands. Since it was discovered and settled by humans in the late 1500s and early 1600s, the island has been subjected to massive habitat destruction, hunting, introduced predators and herbivores, invasive plant species, and human overpopulation (Mauremootoo *et al.* in press). As a result, the island has lost most of its large-bodied vertebrate fauna (Cheke 1987; Cheke & Hume in press), and arguably has one of the most threatened floras of the world. Of 680 original native and endemic plant species, some 80 are already extinct, and 155 are critically endangered. In Mauritius, critically endangered often literally means ‘on the brink of extinction’, as 79 of the critically endangered species are represented by less than ten known individuals, and 12 of these are represented by only a single known individual. A further 93 species are endangered and 241 vulnerable making 82% of the native flora and 94% of the endemic flora of Mauritius threatened according to IUCN criteria (Mauritian Wildlife Foundation, unpublished database).

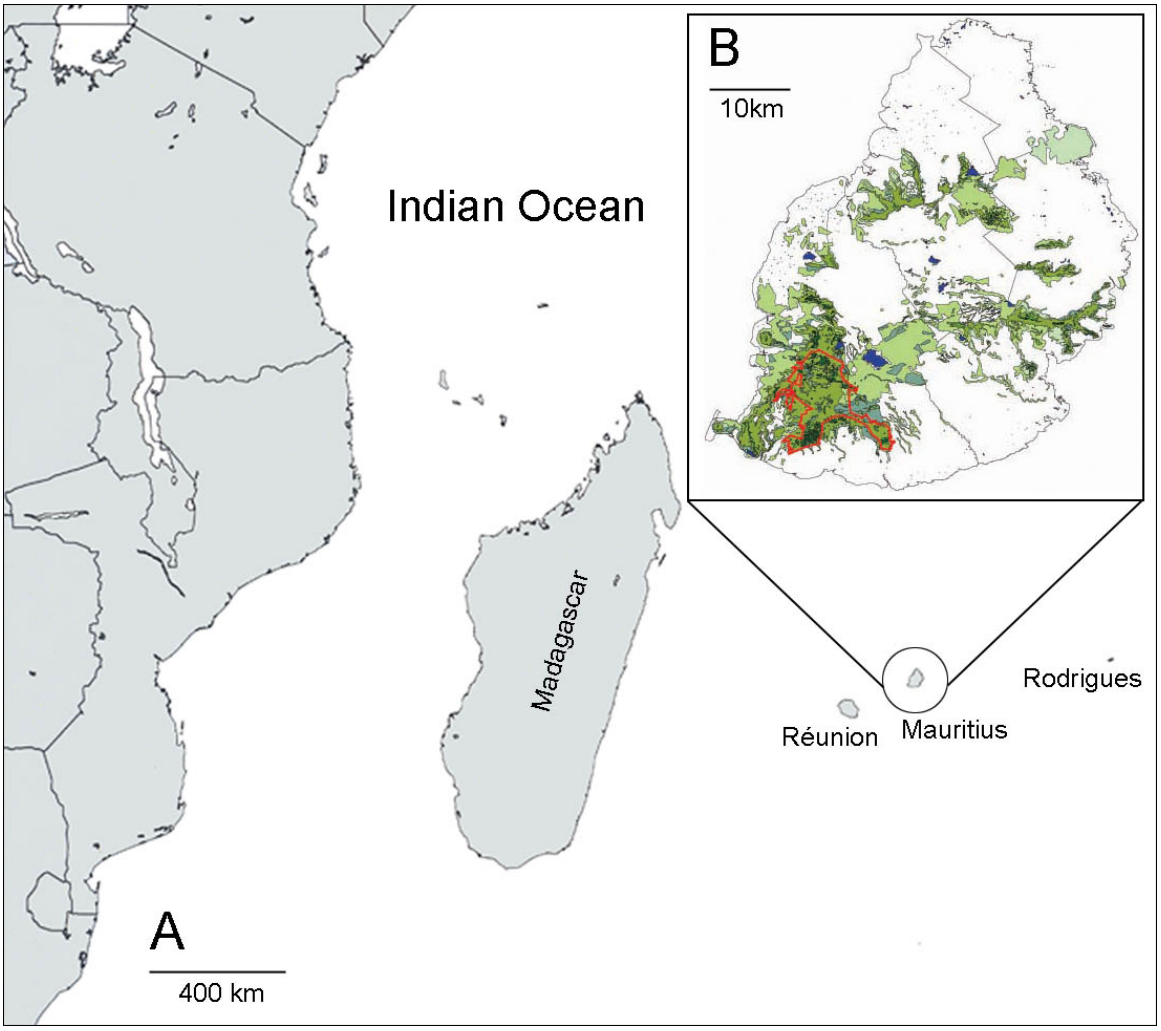



**FIGURE 1.** (A) Part of the western Indian Ocean, showing the Mascarene Islands, Réunion, Mauritius, and Rodrigues, located between 600 and 1300 km east of Madagascar. (B) Map of forest cover in Mauritius. The Black River Gorges National Park is outlined in red. Light green and light blue colours show areas with plantations of exotic trees, medium green areas depict heavily degraded native forest, and dark green areas are the last remnants of native vegetation (forest with >50% native canopy) (Map by C.N. Kaiser; Source inset: Mauritian Wildlife Foundation).

However, Mauritius also exemplifies how much a few dedicated conservation biologists can achieve in rescuing species and habitats from the brink of extinction. Once down to one or a few handfuls of birds each, the Mauritius kestrel *Falco punctatus*, the pink pigeon *Columba mayeri*, and the echo parakeet *Psittacula eques* now each number in their hundreds again after long-term hands-on conservation management, including captive breeding and nest site management. Similarly, some of the most endangered plants are now growing inside fenced and weeded conservation management areas (CMAs), encompassing valuable remnants of native habitat types, where their seedlings stand a

chance of reaching reproductive maturity, and where nursery-grown seedlings are slowly being planted as well.

The initial waves of species extinctions in Mauritius were merely the first harbingers of doom. A much more insidious long-term threat to the maintenance of Mauritian biodiversity is the loss of mutualistic pollination and seed dispersal interactions between plants and animals, and the resulting lack of self-sustaining reproductive dynamics in the native Mauritian forests. Nominally, most of the remaining native habitat in Mauritius is protected today. However, active habitat restoration efforts are currently restricted to small offshore islands and small fenced mainland CMAs, but are likely to expand in area over the next decades, so that several hundred hectares of continuous native forest may be restored (Jones in press). By then, the importance of missing mutualistic partners may become more apparent – i.e. low or no seed set (missing pollinators) and/or little or no dispersal into suitable microhabitats (missing seed dispersers). Most pollination and seed-dispersal mutualisms in the tropics are not specialised relationships (Howe & Smallwood 1982; Bawa 1990). However, with the loss of a large proportion of the native and endemic Mauritian animal mutualists, especially vertebrate seed dispersers, this barely matters. Conservation management of endangered Mauritian plant species must acknowledge this fact, and minimise the negative impacts incurred by the lack of mutualistic partners. Thus, while urgent species-level hands-on conservation is certainly required to stem the immediate flood of extinction events, we must not fail to also address longer-term mitigation of the negative effects caused by the loss of species interactions.

Within the general framework of island biology as outlined above, I specifically address and investigate: (1) the ecology, evolution, and conservation plant–animal interactions on islands, with an emphasis on (2) the importance of mutualistic reptile–plant interactions for endangered plants in Mauritius.

### *Outline of chapters*

When I first went to work as a conservation volunteer on Mauritius in 1998, Olesen *et al.* (1998) had just published on how the "Mauritian coloured nectar remains a mystery". Most floral nectars are clear as water, but here were some species producing blood-red nectar! Olesen and co-workers had stated that there were only three known species with coloured nectar in the world, and that they were all found in Mauritius.

**Chapter 1** clearly demonstrates that this is certainly not the case. It reviews the global taxonomical and geographical distribution of coloured nectar, summarises what is known about the ecology and evolution of coloured nectar, and speculates on its possible functions.

**Chapter 2** returns to Mauritius to investigate the potential function of coloured nectar here. Choice experiments with clear and coloured sugar water in artificial flowers are used to experimentally investigate if coloured nectar in the Mauritian endemic plants can serve as a visual signal for endemic pollinating geckos.

**Chapter 3** continues the lizard pollination theme, and provides the first detailed study of lizard pollination in Mauritius, by studying how the endemic plant *Trochetia blackburniana* is pollinated by endemic *Phelsuma cepediana* geckos. It furthermore investigates how patches of neighbouring non-flowering plants (*Pandanus* spp.) can indirectly structure lizard pollination interactions with flowering plants by creating small-scale microhabitat differences that determine lizard activity levels.

**Chapters 4 and 5** comprise an in-depth study of both the pollination and the seed dispersal ecology of a tropical plant species – in this case the cauliflorous endemic tree *Syzygium mamillatum* (Myrtaceae). The chapters break novel ground in applied conservation biology by investigating the combined importance of (1) habitat restoration for pollination interactions, and (2) the use of ecological analogue seed-dispersing species to replace extinct animals. Furthermore, Chapter 5 provides the first experimental investigation of the Janzen-Connell model for seedling establishment on oceanic islands.

**Chapters 6 and 7** demonstrate the importance of combining basic ecological knowledge and experimental approaches in the conservation of critically endangered island plant species – here the enigmatic endemic Mauritian *Roussea simplex* (Rousseaceae). The basal taxonomical position of *R. simplex* in the order Asterales, combined with an intriguing biogeography, makes the study of the ecology of *R. simplex* important for understanding the evolution and biogeography of Asterales as a whole. **Chapter 6** returns to the importance of lizard–plant interactions on islands by showing that endemic *Phelsuma cepediana* geckos are both pollinators and seed dispersers of *R. simplex*. **Chapter 7** demonstrates how an invasive ant species, *Technomyrmex albipes*, can disrupt both the pollination and the seed dispersal interactions of *R. simplex* by monopolising the use of flowers and fruits.

**Chapter 8** argues that the much-praised approach to improving yields in commercial crops in the tropics, by growing them in proximity of natural forests or other

habitats, can have a negative flip-side because crop pests can invade nearby natural habitats and switch hosts to native or endemic plant species.

All the chapters are written as independent manuscripts for papers. Therefore, there is inevitably considerable overlap between some sections of some chapters. In particular, there is an obvious repetitiveness in the study site sections of the papers. However, it hopefully means that by the end the reader will be left with no doubt about the dire situation that much of the native and endemic biodiversity in Mauritius faces.

***AFFILIATIONS OF COLLABORATORS OTHER THAN PROF. CHRISTINE B. MÜLLER:***

CHRISTOPHER N. KAISER, KARIN BEER, HEINE C. KIESBÜY: Institute of Environmental Sciences, University of Zurich, Switzerland.

JENS M. OLESEN, Department of Ecology & Genetics, Institute of Biology, University of Aarhus, Denmark.

THOMAS MIONE, Biology Department, Copernicus Hall, Central Connecticut State University, New Britain, USA.

STEVEN D. JOHNSON, School of Biological and Conservation Sciences, University of Kwa-Zulu Natal, Pietermaritzburg, South Africa.

CARL G. JONES, Durrell Wildlife Conservation Trust, UK, and Mauritian Wildlife Foundation, Vacoas, Mauritius.

## REFERENCES

BARRETT, S.C.H. (1996) The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society of London - Series B Biological Sciences* 351: 725-733.

BAWA, K.S. (1990) Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399-422.

BERNARDELLO, G., ANDERSON, G.J., STUESSY, T.F. & CRAWFORD, D.J. (2001) A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernandez Islands (Chile). *Botanical Review* 67: 255-308.

BOUCHET, P., JAFFRE, T. & VEILLON, J.M. (1995) Plant extinction in New Caledonia: protection of sclerophyll forests urgently needed. *Biodiversity and Conservation* 4: 415-428.

CARLQUIST, S. (1965) *Island life*. Natural History Press. Garden City, New York.

CARLQUIST, S. (1974) *Island biology*. Columbia University Press. New York.

CHEKE, A.S. (1987) An ecological history of the Mascarene Islands, with particular reference to extinctions and introductions of land vertebrates. In: *Studies of Mascarene Island birds*. pp. 5-89 (Diamond, A.W., ed). Cambridge University Press, Cambridge.

CHEKE, A.S. & HUME, J.P. (in press) *Lost land of the Dodo*. Christopher Helm. London, UK.

CORDEIRO, N.J. & HOWE, H.F. (2001) Low recruitment of trees dispersed by animals in African forest fragments. *Conservation Biology* 15: 1733-1741.

COX, P.A. & ELMQVIST, T. (2000) Pollinator Extinction in the Pacific Islands. *Conservation Biology* 14: 1237-1239.

COX, P.A., ELMQVIST, T., PIERSON, E.D. & RAINEY, W.E. (1991) Flying foxes as strong interactors in South Pacific island ecosystems: a conservation hypothesis. *Conservation Biology* 5: 448-454.

DARWIN, C. (1859) *On the origin of species by means of natural selection*. John Murray. London.

DIAMOND, J. M. (1984) Historic extinctions: their mechanisms, and lessons for understanding prehistoric extinctions. Pp. 824–862 in Quartenary extinctions (Martin, P. S. and Klein, R., eds). University of Arizona Press, Tucson.

DUPONT, Y.L., HANSEN, D.M., VALIDO, A. & OLESEN, J.M. (2004) Impact of introduced honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation* 118: 301-311.

ELMQVIST, T., COX, P.A., RAINEY, W.E. & PIERSON, E.D. (1992) Restricted pollination on oceanic islands: pollination of *Ceiba pentandra* by flying foxes in Samoa. *Biotropica* 24: 15-23.

FRANKHAM, R. (1998) Inbreeding and extinction: Island populations. *Conservation Biology* 12: 665-675.

GODÍNEZ-ÁLVAREZ, H. (2004) Pollination and seed dispersal by lizards: a review. *Revista Chilena de Historia Natural* 77: 569-577.

GRANT, P.R. (1998) *Evolution on islands*. Oxford, UK.

HANSEN, D.M., OLESEN, J.M. & JONES, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography* 29: 721-734.

HNATIUK, S.H. (1978) Plant dispersal by the Aldabran giant tortoise, *Geochelone gigantea* (Schweigger). *Oecologia* 36: 345-350.

HOWE, H.F. & SMALLWOOD, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201-228.

JONES, C.G. (in press) Practical conservation on Mauritius and Rodrigues: steps towards the restoration of devastated ecosystems. In: *Lost land of the Dodo*. (Cheke, A.S., Hume, J.P., eds). Christopher Helm, London.

KEARNS, C.A., INOUYE, D.W. & WASER, N.M. (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29: 83-112.

MACARTHUR, R.H. & WILSON, E.O. (1967) The theory of island biogeography.

MCCONKEY, K.R. & DRAKE, D.R. (2002) Extinct pigeons and declining bat populations: are large seeds still being dispersed in the tropical Pacific? In: *Frugivory and seed dispersal: evolutionary and conservation perspectives* (Levey, D., Silva, W. & Galetti, M., eds), pp. 381–395. CAB International, Wallingford.

MEEHAN, H.J., MCCONKEY, K.R. & DRAKE, D.R. (2002) Potential disruptions to seed dispersal mutualisms in Tonga, Western Polynesia. *Journal of Biogeography* 29: 695-712.

MILBERG, P. & TYRBERG, T. (1993) Naïve birds and noble savages - a review of man-caused prehistoric extinctions of island birds. *Ecography* 16: 229-250.

OLESEN, J.M., RØNSTED, N., TOLDERLUND, U., CORNETT, C., MØLGAARD, P., MADSEN, J., JONES, C.G. & OLSEN, C.E. (1998) Mauritian red nectar remains a mystery. *Nature* 393: 529.

OLESEN, J.M. & VALIDO, A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution* 18: 177-181.

OLSON, S. L. (1989) Extinction on islands: man as a catastrophe. Pp. 50–53 in *Conservation for the twenty-first century* (Western, D. & Pearl, M.C., eds). Oxford University Press, New York.

RICK, C.M. & BOWMAN, R.I. (1961) Galápagos tomatoes and tortoises. *Evolution* 15: 407-417.

RIERA, N., TRAVESET, A. & GARCIA, O. (2002) Breakage of mutualisms by exotic species: the case of Cneorum tricoccon L. in the Balearic Islands (Western Mediterranean Sea). *Journal of Biogeography* 29: 713-719.

SAVIDGE, J.A. (1987) Extinction of an island forest avifauna by an introduced snake. *Ecology* 68: 660-668.

SIMBERLOFF, D. (2000) Extinction-proneness of island species - causes and management implications. *Raffles Bulletin of Zoology* 48: 1-9.

STRAHM, W. (1994) The conservation and restoration of the flora of Mauritius and Rodrigues (PhD Thesis): University of Reading.

TILMAN, D., MAY, R.M., LEHMAN, C.L. & NOWAK, M.A. (1994) Habitat destruction and the extinction debt. *Nature* 371: 65-66.

TRAVESET, A. (1999) The importance of mutualisms for biodiversity conservation in insular ecosystems. *Revista Chilena de Historia Natural* 72: 527-538.

TRAVESET, A. & RICHARDSON, D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution* Online Early.

TRAVESET, A. & RIERA, N. (2005) Disruption of a plant-lizard seed dispersal system and Its ecological effects on a threatened endemic plant in the Balearic Islands. *Conservation Biology* 19: 421-431.

VALIDO, A. & OLESEN, J.M. (In press) Importance of lizards as frugivores and seed dispersers. In: *Seed dispersal: theory and its application in a changing world*. (Dennis, A., Green, R., Schupp, E., Westcott, D., eds). CABI Publishing.

VITOUSEK, P.M., LOOPE, L.L. & ADSERSEN, H. (1995) *Islands: biological diversity and ecosystem function*. Springer Verlag. Heidelberg, Germany.

WALLACE, A.R. (1869) *The Malay Archipelago: The land of the Orang-Utan and the Bird of Paradise*. Macmillan. London.

WALLACE, A.R. (1880) *Island life*. Macmillan. London.

WHITTAKER, R.J. (1998) *Island biogeography. Ecology, evolution, and conservation*. Oxford University Press. Oxford.

# CHAPTER 1

**Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait**

There are few flowers that do not ſecrete from ſome kind of a glandular ſubſtance, honey, or nectar, to a greater or ſmaller amount; in thoſe of the preſent genus, this liquid is particularly abundant, even dropping from the flowers of the *major*, in conſiderable quantity; in the preſent ſpecies it flows not ſo copiouſly, but is retained in the lower part of the bloſſom, and is of a dark brown colour, an unuſual phenomenon.

On *Melianthus comosus* in Curtis's Botanical Magazine, 1795

*Biol. Rev.* (2007), **82**, pp. 83–111.
doi:10.1111/j.1469-185X.2006.00005.x


# Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait

Dennis M. Hansen[1]*, Jens M. Olesen[2], Thomas Mione[3], Steven D. Johnson[4] and Christine B. Müller[1]

[1] *Institute of Environmental Sciences, University of Zurich, Winterthurerstrasse* 190, 8057 *Zurich, Switzerland*
[2] *Department of Ecology & Genetics, University of Aarhus, Block* 540, *Ny Munkegade,* 8000 *Aarhus C, Denmark*
[3] *Biology Department, Copernicus Hall, Central Connecticut State University, New Britain, CT* 06050-4010, *USA*
[4] *School of Biological and Conservation Sciences, University of KwaZulu-Natal, P. Bag X*01 *Scottsville, Pietermaritzburg* 3209, *South Africa*

(*Received* 17 *March* 2006; *revised* 25 *October* 2006; *accepted* 6 *November* 2006)

ABSTRACT

While coloured nectar has been known to science at least since 1785, it has only recently received focused scientific attention. However, information about this rare floral trait is scattered and hard to find. Here, we document coloured nectar in 67 taxa worldwide, with a wide taxonomical and geographical distribution. We summarise what is currently known about coloured nectar in each of the lineages where it occurs. The most common nectar colours are in the spectrum from yellow to red, but also brown, black, green, and blue colours are found. Colour intensity of the nectar varies, sometimes even within one taxa, as does the level of contrast between flower petals and nectar. Coloured nectar has evolved independently throughout the angiosperms at least 15 times at the level of family, and is in many cases correlated with one or more of three parameters: (1) vertebrate pollination, known or hypothesised, (2) insularity – many species are from islands or insular mainland habitats, and (3) altitude – many species are found at relatively high altitudes. We discuss the evolution and speculate on possible ecological functions of coloured nectar. Apart from being a non-functional, perhaps pleiotropic, trait, we present several hypotheses on possible ecological functions of coloured nectar. Firstly, for some plant species it can be interpreted as an honest signal, leading to high pollination efficiency. Secondly, it can function as a deterrent against nectar-thieves or inefficient pollinators, thus acting as a floral filter. Thirdly, nectar colour-pigments can have anti-microbial qualities that may protect the nectar in long-lived flowers. Neither of these possibilities are mutually exclusive. Recent studies have provided experimental evidence for the first two hypotheses, and we suggest promising avenues for future research into this little-known floral trait.

*Key words*: floral trait, nectar properties, mutualism, pollination biology, pollinator attraction, signalling theory, honest signal, floral filter.

CONTENTS



* Address for correspondence: E-mail: dhansen@uwinst.unizh.ch

## I. INTRODUCTION

Ever since Sprengel's landmark publication in 1793, researchers have tried to understand how floral traits affect interactions with pollinators, and *vice versa*. One floral trait that has received much attention is nectar, the main 'currency' of many pollination interactions. Analytical research on nectar properties has focused on the composition of sugars (Percival, 1961; Baker & Baker, 1983; Baker, Baker & Hodges, 1998) and amino acids (Baker & Baker, 1973, 1986; Gottsberger, Schrauwen & Linskens, 1984). Lately, more specific ecological traits and functions of nectar have been studied; e.g. taste (Gardener & Gillman, 2002), toxicity (Ehlers & Olesen, 1997; Adler, 2000), and scent (Raguso, 2004b), including how these traits interact with the physiology and behaviour of flower-visiting animals. The general conclusions in these studies are that little is known about 'nectar ecology'. Herein we review the occurrence of a particularly enigmatic nectar trait, colour, and speculate about its ecological functional significance and evolution.

A divine liquid in mythology, red nectar is known from antiquity. In the Illiad, Homer describes the actions of the goddess Thetis as she preserves the body of the dead warrior Patroclus: "she then dropped ambrosia and red nectar into the wounds of Patroclus, that his body might suffer no change." (Book XIX, verses 37–39). In modern science, coloured nectar has a long history as well. The first reference to coloured nectar is found in *Dissertatio de Aloe* (Hesselius, 1785), where the description of *Aloe spicata* includes the line, "*Corollae repletae sunt succo melleo purpurascente*", clearly referring to the corolla being replete with sweet, dark-red nectar. The most eloquent early description of coloured nectar is found in one of the oldest volumes of Curtis's Botanical Magazine (1795, Volume 9, Plate 301), where the description of *Melianthus minor* (= *M. comosus*) (Melianthaceae) states: "There are few flowers that do not secrete from some kind of a glandular substance, honey, or

nectar, to a greater or smaller amount; in those of the present genus, this liquid is particularly abundant … and is of a dark brown colour, an unusual phenomenon''. Furthermore, the accompanying plate shows this detail in such quality, that it is possible to see the dark nectar through the pale green, semi-transparent sepals, much like it can be seen in the wild (Fig. 1). This species was first described only one year earlier, but the diagnosis does not mention the coloured nectar, or even the abundance of nectar (Vahl, 1794). This points to the crux of the matter in our current understanding of coloured nectar: for the majority of taxa with coloured nectar, the taxonomical description does not mention the colouration of the nectar. Another early example of this is *Jaltomata aspera* (Solanaceae) of Peru. Ruiz & Pavón (1799) described the pale cream-coloured corolla of *J. aspera*, as ''violet in the centre''. However, long reflected in its local name, *lágrima de la virgen*, or 'tear of the virgin', the red nectar of this plant was not described by botanists until Bitter (1921) wrote about the ''copious production of a blood-red juice from glands at the bottom of the corolla between the anthers''. In the case of *Melianthus* and *Aloe*, where coloured nectar has long been known, it has not been reported in mainstream ecological or botanical journals, but only in taxonomical descriptions and studies. In most other lineages, the coloured nectar is not even mentioned in the taxonomical descriptions or studies of the plants.

Given its visual impact, it is surprising that so little is known about coloured nectar. The almost complete omission of reference to nectar colour in the taxonomical literature has limited our knowledge of the geographical and taxonomical distribution of coloured nectar, and has made studies of its possible ecological function and evolution difficult. The main reason is without doubt that the original descriptions were based on herbarium material. Here, of course, the nectar has long dried out, leaving at most only discoloured marks that are difficult to interpret. Marloth (1925), for example, simply discounted the dark stains of nectar on a dry herbarium specimen of *A. spicata* as a reaction between clear nectar and the filaments or the paper used in pressing the plant – a mistake that was later pointed out by Glen & Hardy (1995). To summarise, in all taxa coloured nectar was only mentioned in the literature after investigation of living plants.


**Fig. 1.** *Melianthus minor* flowers. (A) Part of an illustration in Curtis's Botanical Magazine (1795, Volume 9, Plate 301). (B) Photograph of flowers of a botanical garden plant. Note how the black nectar is clearly visible through the pale, semi-translucent sepals in both pictures. Photograph by W. Barthlott.

*Nesocodon mauritianus* (Campanulaceae) was the first species with coloured nectar for which the ecology and nectar-chemistry was studied in detail (Olesen *et al.*, 1998). The species was discovered on the island of Mauritius in 1976, but there was no mention of the blood-red colour of the nectar in the taxonomical description (Richardson, 1979). Later, after studying *N. mauritianus* plants in cultivation, Wyse Jackson (1990) was the first to mention the red nectar of this species. Olesen *et al.* (1998) also reported the presence of coloured nectar in two Mauritian *Trochetia* (Malvaceae) species, *T. blackburniana* and *T. boutoniana*, and stated that – to their knowledge – these plants, along with *N. mauritianus*, were the only ones in the world with coloured nectar. From our detailed research for this review it is now obvious that this is not true. However, information on coloured nectar is hard to find in the mainstream scientific literature, and is often known only from grey literature or observations. Our study reviews the occurrence of and knowledge on coloured nectar in flowering plants. Specifically, we aim to (1) document and investigate the geographical and taxonomical distribution of coloured nectar in flowering plants, (2) summarise the current knowledge about species with coloured nectar and the lineages in which they occur, (3) investigate possible environmental and ecological correlates of coloured nectar, and (4) discuss its possible ecological functions and its evolution.

## II. METHODS

We here define coloured nectar as a floral sugary secretion that contains one or more pigments or coloured substances that are apparently produced and secreted by the plants. We include only species with nectar colours in the spectrum visible to humans. One study has documented ultraviolet-fluorescent nectar in several species (Thorp *et al.*, 1975). However, there have been no further studies of this trait, and we have not included species with known UV-fluorescent nectar in our study.

The idiosyncratic literature on coloured nectar precluded any attempt to obtain data in a standardised and methodological way. We conducted a thorough search of the literature, using library and scientific databases, web pages, scientific publications, and regional and national floras. In web-based databases and search engines we used specific queries, searching for 'nectar' in combination with each of the following words 'colour, coloured, dark, black, red, brown, yellow, orange, green, blue, purple' in English (US and UK), Spanish, Portuguese, French, German and Danish. Furthermore, we contacted many pollination biologists, plant biologists and taxonomists, attempting to cover a broad geographical and taxonomical range, and inquired about observations of coloured nectar. When coloured nectar was confirmed for a species, we also acquired information from the literature about related species, and by contacting researchers studying related species within the same lineage. We also included our personal observations on taxa with coloured nectar. We compiled a database with data on growth form, geographical

and taxonomical distribution and altitudinal range; on floral traits, including size, colour and morphology; and on nectar traits, including volume, concentration and sugar composition. Then we examined this database, aiming to identify any potential correlations that would help us formulate hypotheses on its possible ecological functions and evolution of coloured nectar. Furthermore, we gathered information on the different lineages with coloured nectar and their closest relatives to summarise our current knowledge within each lineage. If available, we included ecological and evolutionary information in our summary.

## III. RESULTS

### (1) Taxonomical and geographical distribution

In total, we were able to document the presence of coloured nectar in 68 taxa from 20 genera in 15 families (Table 1; Fig. 2). These taxa occur in tropical and subtropical regions, with a wide geographical distribution (Table 1). We found that coloured nectar occurs in at least 13 angiosperm orders, covering widely separated branches of the phylogenetic tree. Coloured nectar is found in both monocots and eudicots, but not in the magnoliids or paleoherbs (Fig. 3).

### (2) Flower and nectar traits

The flowers of species with coloured nectar show large variation in nectar colours (Table 1, Fig. 2). The colours of nectar can be divided into the following broad categories (using the 'darkest' recorded nectar colour for a taxon in Table 1; relative frequency in parenthesis): yellow (15 taxa; 22.1%), amber – orange (six taxa; 8.8%), red (17 taxa; 25%), brown (14 taxa; 20.6%), green (five taxa; 7.4%), blue (two taxa; 2.9%) and black (nine taxa; 13.2%). Thus, we find that 55.9% of the taxa have nectar colours in the range from yellow to red, and most of those with darker colours (brown or black) are very dark hues of red. Only in *Schiedea* does the black colouration seem to originate from grey hues (S. G. Weller, personal communication). The green colours of the nectar in some *Banksia* species are also clearly outside the 'red' group, even though the nectar in young flowers of these species has a yellow colouration (Markey & Lamont, 1995). *Puya alpestris* and *Schwartzia brasiliensis* are the only known species with blue or blue-red nectar. The level of contrast between the flower petals and the nectar also varies (Table 1). In most species, the nectar contrasts well against the petals in the human visible spectrum, but even in plants with overall petal colours similar to the nectar colour, the nectar often still contrasts due to being present on lighter parts of the corolla (see Fig. 2, and Section IV.4).

Species with coloured nectar also vary widely in growth form and floral morphology, with plants ranging from vines and small shrubs to large trees, and having from radially symmetrical to strongly zygomorphic flowers in many different colours (Table 1; Fig. 2). However, most flowers are open and easily accessible to visitors (e.g. *Hoya*, Fig. 2B,C; *Trochetia* Fig. 2G; *Aloe* Fig. 2D, J; *Capsicum* Fig. 2P), or if more closed or tubular, the nectar can often be seen from the outside at a distance, through a pale or semi-translucent calyx or corolla (in some *Melianthus* species, Fig. 1; and in some *Jaltomata* species, Fig. 2K). For nectar traits, we have only found information for a subset of the plants with coloured nectar (Table 2). From this it is clear, however, that most produce relatively large amounts of nectar, forming visible drops in the flowers. Furthermore, most of the species for which we have information have sugars that are hexose-dominated (Table 2).

### (3) Lineage descriptions

In the following we summarise and discuss our current knowledge of coloured nectar in each lineage, with an emphasis on pollination ecology if data are available.

#### (a) *Asparagales* – *Asphodelaceae* – Aloe

*Aloe* is a large genus with some 365–400 species, native to Africa, the Arabian Peninsula and Madagascar (Mabberley, 1997; Viljoen, van Wyk & van Heerden, 1998). Growth forms vary from dwarf rosettes to trees of more than 15 m in height. Most *Aloe* species have large inflorescences with long, slender, pendulous and tubular flowers (van Wyk & Smith, 1996). However, one small section, *Anguialoe*, comprises four species with very dense racemes or spikes that carry short, sessile and campanulate flowers (Reynolds, 1940; Glen & Hardy, 1995). Coloured nectar is found in all four species currently recognised within *Anguialoe*, albeit at varying levels of colour intensity (Fig. 2D, J; Table 1). Contrary to most other lineages where coloured nectar occurs, it has been known for a long time in *Aloe*. Hesselius' (1785) treatment of the genus contains the first scientific reference to coloured nectar that we have been able to find (see Section I), and it has been mentioned regularly in the scientific literature since then. For example, Pole Evans (1925) describes the flowers of *A. sessiliflora* (= *A. spicata*), stating that they "contain drops of very dark nectar", and Groenewald (1938), in his description of *A. dolomitica* (= *A. vryheidensis*), makes a note of the dark brown nectar in the flowers. Dyer (1931) mentions the flowers of *A. sessiliflora* being "filled with a reddish nectar", and later he states that the nectar of *A. vryheidensis* "appears almost black in colour" (Dyer, 1941). In the original circumscription of *Anguialoe*, Reynolds (1940) even used the flowers' "copious supply of dark nectar" as a defining trait for the section. However, clear rather than coloured nectar has been noted in the wild for *A. alooides* (B.-E. van Wyk, personal communication), but in a greenhouse in the National Botanical Garden of Belgium, a plant produced red nectar in 2002 (B. Loison, personal communication). Lastly, the dark nectar is also mentioned in some of the more popular accounts of *Aloe* species (e.g. Court, 1981; van Wyk & Smith, 1996). For *Aloe* section *Anguialoe* in general, Reynolds (1940) remarked that the nectar seems to be of a lighter colour in young flowers, while it is darker in the oldest flowers. Nicolson & Nepi (2005) mention the same for the nectar in *A. castanea* flowers. This colour change is due to oxidation of phenolic compounds that impart the dark brown colour to the nectar (H. F. Glen, personal communication; S. D. Johnson, A. Hargreaves & M. Brown, unpublished data). A recent study has shown that *Aloe*

Table 1. Plant taxa with coloured nectar; their geographical and altitudinal distribution, growth form, flower morphology, nectar colour and observed flower visitors ('nd' = no data available)

| Order | Family | Species | Distribution | Altitude (masl) | Growth form | Flower form | Flower size (mm)[1] | Flower colour[2] | Nectar colour[2] | Flower visitors[3] |
|---|---|---|---|---|---|---|---|---|---|---|
| Asparagales | Asphodelaceae | *Aloe alooides* | South Africa | 1450–2000 | shrub | campanulate | 9 | yellow | clear – red | B, I |
| | | *A. castanea* | South Africa | 1400–1700 | shrub | campanulate | 18–19 | orange – red | yellow – brown | B, I |
| | | *A. vryheidensis* | South Africa | 1300–1550 | shrub | campanulate | 14 | yellow | dark red – brown | B, I |
| | | *A. spicata* | South Africa, Zimbabwe | 700–1000 | shrub | campanulate | 18–20 | yellow | dark red – brown | B, I |
| | Hemerocallidaceae | *Phormium tenax* | New Zealand | 0–1000 | shrub | tubular | 26 × 7–9 | orange – red | clear – yellow | B, L |
| Asterales | Campanulaceae | *Nesocodon mauritianus* | Mauritius | 5–600 | subshrub | campanulate | 50 × 30 | blue | orange – red | B?, L?, B† |
| Caryophyllales | Caryophyllaceae | *Schiedea lychnoides* | Kaua'i | 1090–1320 | vine | campanulate | 5, 9–12[4] | white[5] | black | u, B? |
| | | *S. obovata* | O'ahu | 550–800 | shrub | campanulate | 7–8.4[4] | white[5] | black | u, B? |
| | | *S. trinerve* | O'ahu | 900–1230 | subshrub | ball-shaped | 6–8[4] | white[5] | black | u, B? |
| | | *S. viscosa* | Kaua'i | 820–1150 | vine | campanulate | 5, 6.5–9[4] | white[5] | black | u, B? |
| Ericales | Marcgraviaceae | *Schwartzia brasiliensis* | Brazil | 0-800 | vine | urceolate[6] | 10–12 × 8–10[6] | dark purple-brown[6] | blue | B, I |
| Fabales | Fabaceae | *Calliandra calothyrsus* | S Mexico to C Panama | 0–1800 | tree | brush-type | 6–8, 40–60[7] | green and red[8] | yellow | M, I, B |
| | | *Erythrina caffra* | South Africa | nd | tree | zygomorphic | 42–70 × 27–40 | red | clear – pale brown | B |
| | | *E. humeana* | Southern Africa[9] | nd | shrub | zygomorphic | 35–50 × 14–21 | red | amber | B |
| | | *E. zeyheri* | South Africa | 1700–1750 | subshrub | zygomorphic | 24–44 × 14–22 | red | pale yellow | B |
| Gentianales | Apocynaceae | *Hoya diversifolia* | Asia[10] | lowland | vine | rotate | 13 (diameter) | cream – pale pink[11] | amber – brown | u |
| | | *H. excavata* | Malaysia | nd | vine | rotate | 13 (diameter) | pink[11] | brown | u |
| | | *H. kerrii* | Asia[10] | 390 | vine | rotate | 9–13 (diameter) | white – cream[11] | amber – brown | u |
| | | *H. meliflua* | Philippines | lowland | vine | rotate | 15 (diameter) | pink – purple[11] | dark red | u |
| | | *H. obovata* | Asia[10] | nd | vine | rotate | 15 (diameter) | cream – pink[11] | brown | u |
| Geraniales | Melianthaceae | *Melianthus comosus* | South Africa, Namibia | 400–2000 | shrub | zygomorphic | 15–32 × 10[12] | green – pale pink[13] | black | B, I |
| | | *M. dregeanus* | South Africa | 600–1800 | shrub | zygomorphic | 15–20 × 10[12] | red[13] | brown | B |
| | | *M. elongatus* | South Africa | 0–300 | shrub | zygomorphic | 15–22 × 8[12] | green[13] | black | B |
| | | *M. gariepinus* | South Africa, Namibia | 400–2000 | shrub | zygomorphic | 15–22 × 7–10[12] | green[13] | brown | B |
| | | *M. insignis* | South Africa | 900–1800 | shrub | zygomorphic | 15–40 × 10[12] | red[13] | brown | B |
| | | *M. major* | South Africa | 300–900 | shrub | zygomorphic | 15–35 × 10–12[12] | dark red – brown[13] | brown | B |
| | | *M. pectinatus* | South Africa | 0–900 | shrub | zygomorphic | 10–17 × 5–10[12] | green[13] | black | B |
| | | *M. villosus* | South Africa | 1600–2000 | shrub | zygomorphic | 15–35 × 10–12[12] | purple – brown[13] | black | B, I |
| Lamiales | Bignoniaceae | *Deplanchea tetraphylla* | New Guinea, Australia | 0–1000 | tree | zygomorphic | 25 × 25 × 10[14] | yellow | amber – dark brown | B, M |
| | | *Fernandoa magnifica* | Eastern Africa[15] | 0–500 | tree | broadly campanulate | 50–110 | yellow – red | dark brown – black | B?, M? |

Table 1 (*cont.*)

| Order | Family | Species | Distribution | Altitude (masl) | Growth form | Flower form | Flower size (mm)[1] | Flower colour[2] | Nectar colour[2] | Flower visitors[3] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lamiaceae | *Leucosceptrum canum* | Himalaya to China[16] | 610–2600 | shrub – small tree | campanulate | 8–10 × 6 | white – cream | dark brown | B, I |
| Malvales | Malvaceae | *Dombeya a.* ssp. *acutangula* | La Réunion | 0–1200 | shrub – small tree | broadly campanulate | 20–30 (diameter) | white – cream | yellow | B, I† |
| | | *D. a.* ssp. *palmata* | La Réunion | nd | shrub – small tree | broadly campanulate | 20–25 (diameter) | white – cream | yellow | B, I, I† |
| | | *D. a.* ssp. *rosea* | Mauritius | 200–600 | shrub – small tree | broadly campanulate | 20–25 (diameter) | pink | yellow | B, I, I† |
| | | *D. cacuminum* | Madagascar | montane forests | tree | campanulate | 40 | red | yellow | M, B? |
| | | *D. elegans* | La Réunion | 800–1800 | shrub – small tree | campanulate | 10–14 | pink | yellow | B |
| | | *D. kefaensis* | Ethiopia | 2200 | shrub | campanulate | 13–17 | white – pale pink | red | u |
| | | *Trochetia blackburniana* | Mauritius | 300–700 | shrub – small tree | campanulate | 15–25 × 18–25 | pale pink – red | clear – amber | B, L, I† |
| | | *T. boutoniana* | Mauritius | 400–600 | shrub – small tree | campanulate | 45–50 × 45–50 | pink – red | orange – red | B, L, I† |
| | | *T. granulata* | La Réunion | 1200–1600 | shrub | campanulate | 36–41 × 29–32 | white | yellow | B, I† |
| | | *T. parviflora* | Mauritius | 300–600 | shrub | crateriform rotate | 8–11 × 18–21 | white – pale pink | clear – yellow | u, I? |
| | | *T. triflora* | Mauritius | 500–700 | shrub – tree | broadly campanulate | 25–40 × 50–70 | white | yellow | B, I†, M? |
| | | *T. uniflora* | Mauritius | 400–700 | shrub | campanulate | 25–30 × 19–28 | pink – red | orange – red | B, I† |
| Myrtales | Combretaceae | *Lumnitzera littorea* | Guam[17] | lowland, coastal | shrub – tree | campanulate | 8–10 × 8–10[18] | red | orange | B, I |
| Poales | Bromeliaceae | *Puya alpestris* | Chile | 1000[19] | shrub | tubular-campanulate | 50 | blue | pale pink – blue | B, I |
| Proteales | Proteaceae | *Banksia grossa* | SW Australia | nd | shrub | zygomorphic | 34–45 | brown | yellow – green | I, M, B? |
| | | *B. incana* | SW Australia | nd | shrub | zygomorphic | 21–23 | yellow | yellow – green | I, M, B? |
| | | *B. leptophylla* | SW Australia | nd | shrub | zygomorphic | 35–45 | yellow – brown | yellow – green | I, M, B |
| | | *B. nutans* | SW Australia | nd | shrub | zygomorphic | 22–33 | purple – brown | yellow | I, M, B? |
| | | *B. sphaerocarpa* | SW Australia | nd | shrub | zygomorphic | 24–39 | brown | yellow – green | I, M, B? |
| | | *B. telmatiaea* | SW Australia | nd | shrub | zygomorphic | 22–25 | orange – pale brown | yellow – green | I, M, B? |
| | | *Grevillea robusta* | E Australia | 0–1120 | tree | zygomorphic | 23 | yellow – red | pale yellow – red | B, M?, I† |
| Solanales | Solanaceae | *Capsicum baccatum* | South America[20] | 500–1500 | vine – shrub | rotate | 3.5–7[21] | white – cream | clear – yellow | u |
| | | *C. pubescens* | Bolivia[22] | 1200–2000 | vine – shrub | rotate | nd | purple, white | clear – yellow | I |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *C. eximium* | Bolivia, Argentina | 1500–3000 | vine | rotate | 20–25 (diameter) | purple, white | clear – yellow | u |
| *Jaltomata aspera* | Peru | 0–500 /1600–2550[23] | subshrub | crateriform rotate | 49 (diameter) | pale yellow-green | red | u, B? |
| *J. biflora* | Peru | 2700–3200 | shrub | urceolate | 13 × 14 | pale green | clear – orange | u. B? |
| *J. contumacencis* | Peru | 2840 | shrub | campanulate-tubular | 10 × 25–28 | pale green | clear – orange | u, B? |
| *J. herrerae* | Peru, Bolivia | 3000–3800 | shrub | campanulate | 15–20 × 35–45 | pale green | red | u, B? |
| *J. leviae* | Peru | 2530–3000 | vining shrub | urceolate-tubular | 12 × 18–19 | red, blue – purple[24] | red | u, B? |
| *J. paneroi* | Peru | 3200–3550 | shrub | campanulate | 5–10 × 23–25 | pale green | red | u, B? |
| *J. umbellata* | Peru | 0–500 | shrub | tubular, rotate limb | 9–11 × 14–23 | cream – pale green | red | u, B? |
| *J. ventricosa* | Peru | 2500–3200 | shrub | urceolate | 8–10 × 12–14 | white – pale yellow | orange – red | u, B? |
| *J. weberbaueri* | Peru | 3300–3700 | subshrub | broadly campanulate | 40–45 × 55–60 | pale green – violet | red | u, B?, I? |
| *J.* sp. *'642'*[25] | Peru | 2630–2650 | shrub | urceolate | 7–9 × 14 | pale green | red | u, B? |
| *J.* sp. *'647'*[25] | Peru | 3400–3530 | shrub | broadly campanulate | 19–27 × 32–36 | pale green – purple | clear – orange | u, B? |
| *J.* sp. *'669'*[25] | Peru | 2840 | shrub | campanulate-tubular | 10 × 25–28 | pale green | red | u, B? |
| *J.* sp. *'711'*[25] | Peru | 1420–1870 | shrub | campanulate | 10 × 28 | green | orange – red | u, B? |

[1] Perianth length, or perianth length × diameter, or as noted.
[2] A dash between two colours indicates an approximate continuous range.
[3] Hypothesised flower visitors from the literature or from personal communications are indicated with a '?', non-native flower-visitors are indicated with a '†'. Flower visitor codes: u = unknown, B = birds, I = insects, L = lizards, M = mammals.
[4] Sepal length (Wagner *et al.*, 2005).
[5] White is the colour of the petaloid sepals.
[6] Refers to the cup-shaped nectary; the flowers are red-brown, rotate and 6–8 mm in diameter.
[7] First set of numbers refers to corolla length, second set to length of staminal filaments.
[8] The small petals are green, while the numerous long staminal filaments are red.
[9] South Africa, Mozambique and Zimbabwe.
[10] Asian distributions: *H. kerrii* is found in China, Cambodia, Laos, S. Vietnam, S. Thailand and Java; *H. obovata* in India, Indonesia, Thailand and Fiji; and *H. diversifolia* in Cambodia, Laos, Myanmar, S. Vietnam, Malaysia, Singapore, Thailand and Indonesia.
[11] Colour refers to corolla; the prominent central corona is pink – purple in all species (see Fig. 2B,C).
[12] Size of outer sepals.
[13] Colour refers to the large sepals; the small petals are red or orange, but are mostly hidden inside the sepals (however, see main text on *Melianthus*).
[14] Length, height and diameter of corolla, respectively.
[15] Malawi, Mozambique, Zimbabwe, Kenya, Tanzania.
[16] Bhutan, China, India, Laos, Myanmar, Nepal, Vietnam.
[17] Guam is the only place where coloured nectar has been observed in this species. The total distribution is very wide; ranging from East Africa to Australia and the Western Pacific, but nothing is known about nectar colour in these regions.
[18] Diameter × depth of flower cup, including the part formed by the calyx.
[19] Average altitude for coastal hill and Andean.
[20] Distribution of the wild progenitor: Bolivia, Argentina, Peru, Paraguay, Brazil.
[21] Length of corolla lobe.
[22] Most likely origin of wild progenitor.
[23] Lower range is from the coastal Lomas habitat, higher range from the Andes Mountains.
[24] Tube is red, limb is blue – purple.
[25] Numbers refer to accessions by Mione, Leiva and Yacher.

*vryheidensis*, one of the species with dark brown nectar, is effectively pollinated by a variety of non-specialised nectarivorous birds, including white-eyes, bulbuls and rock thrushes (Johnson, Hargreaves & Brown, 2006; Fig. 2R). Earlier anecdotal records indicate that pollination by these occasional nectarivores also occurs in other members of *Aloe* section *Anguialoe* (Skead, 1967; Cheke & Mann, 2001). The experiments conducted by Johnson *et al.* (2006) showed that artificial

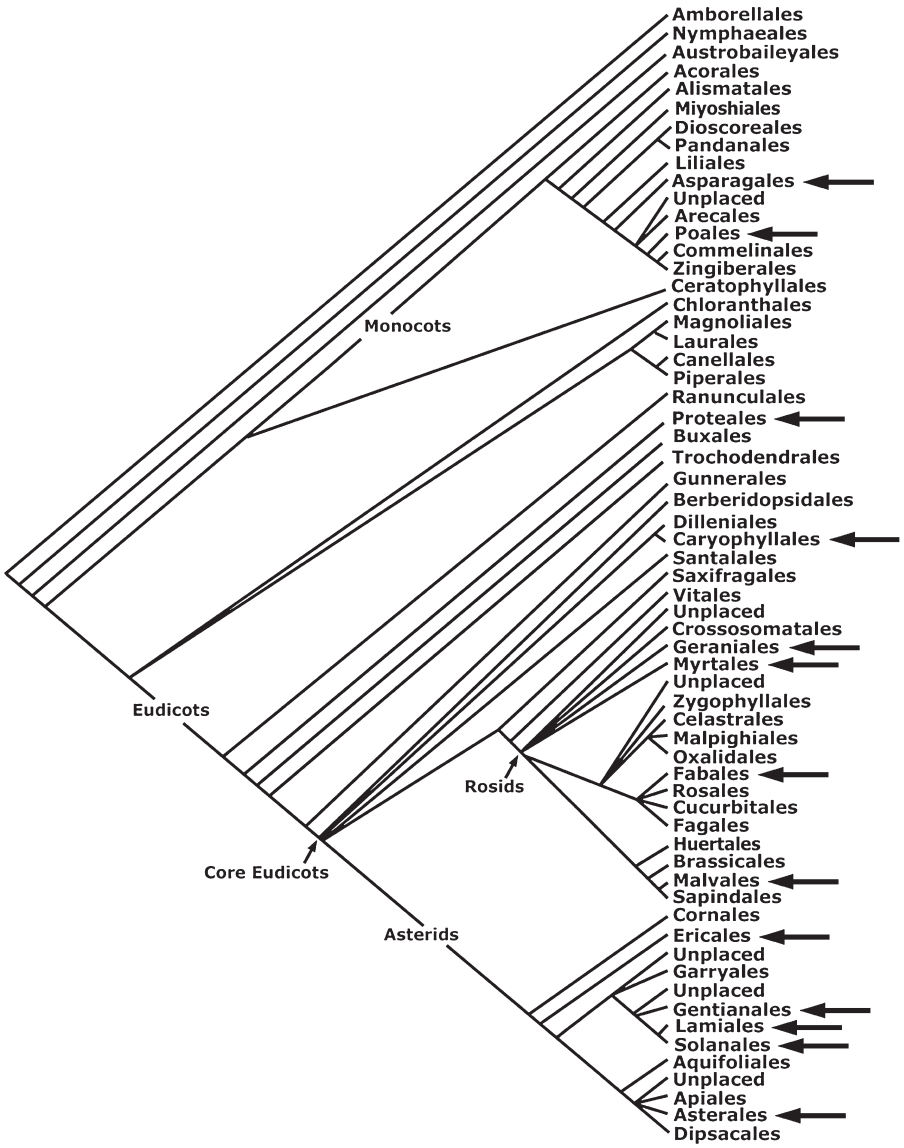



**Fig. 3.** Taxonomical distribution of coloured nectar in the angiosperms at the level of order. Arrows indicate the presence of one or more taxa with coloured nectar in the respective orders (see Table 1 for details). Figure modified from Stevens (2001 onwards).

flowers with darkened nectar receive more probes by black-capped bulbuls than do artificial control flowers with clear nectar. However, the main reason why specialised nectarivores such as sunbirds do not visit *A. vryheidensis* is that they find the phenolics in the nectar highly distasteful. Upon probing the flowers and sampling the nectar, these birds would rapidly retract their beaks, shaking their heads vigorously, and not probe such flowers again. Honeybees collect pollen from flowers of *A. vryheidensis*, but find the nectar too unpalatable to consume (Johnson *et al.*, 2006).

**Fig. 2.** Flowers of plant species with coloured nectar, illustrating the wide variety in flower form, colour and nectar colour. (A) *Nesocodon mauritianus* (Campanulaceae). (B) *Hoya diversifolia* (Apocynaceae), single flower, side view. (C) *Hoya diversifolia*, inflorescence. (D) *Aloe vryheidensis* (Asphodelaceae). (E) *Dombeya angulata* ssp. *rosea* (Malvaceae) – note also the secondary pollen presentation on the tip of the petals. (F) *Schwartzia brasiliensis* (Marcgraviaceae). (G) *Trochetia blackburniana* (Malvaceae). (H) *Schiedea obovata* (Caryophyllaceae). (I) *Grevillea robusta* (Proteaceae). (J) *Aloe castanea*. (K) *Jaltomata umbellata* (Solanaceae). (L) *Jaltomata weberbaueri*. (M) *Jaltomata paneroi*. (N) *Deplanchea tetraphylla* (Bignoniaceae). (O) *Fernandoa magnifica* (Bignoniaceae). (P) *Capsicum pubescens* (Solanaceae). (Q) Male *Phelsuma cepediana* gecko nectar feeding in a *Trochetia blackburniana* flower. (R) *Zosterops pallidus* nectar feeding on an *Aloe vryheidensis* inflorescence. Photographs by J. M. Olesen (A, M), P. Wallin (B), K. F. Yap (C), W. Barthlott (D), D. M. Hansen (E, G, Q), I. & M. Sazima (F), N. Kawakubo (H), N. Eden (I), M. Nepi (J), T. Mione (K), S. Leiva G. (L), A. Weber (N), C. Puff (O), A. Boatman (P), S. D. Johnson (R).

Table 2. Nectar properties of plant taxa with coloured nectar ('nd' = no data available)

| Species | Nectar volume[1] (μl) | Sugar concentration[1] (%) | Sugar composition[2] |
|---|---|---|---|
| *Aloe castanea* | 17–100[3] | 6–18[3] | 0.020 – HD |
| *A. vryheidensis* | 27–70 | 6–17 | nd |
| *Phormium tenax* | 135–166[3] | 15.8–20.3[3] | nd |
| *Nesocodon mauritianus* | 2.2 ± 0.86[4] | 11–25 | HD |
| *Schiedea lychnoides* | 18.3 | nd | HR |
| *S. obovata* | 16.6 | nd | HR |
| *S. trinerve* | 30.9 | nd | HR |
| *S. viscosa* | 6.3 | nd | HR |
| *Schwartzia brasiliensis* | 100–150 | 12.6 (5–20) | 0.09 – HD |
| *Calliandra calothyrsus* | 5–55 | nd | nd |
| *Erythrina humeana* | nd | 14.2 | 0–0.042 – HD |
| *E. caffra* | 300 | 5.5–10 | 0–0.042 – HD |
| *Melianthus comosus* | 41.7 ± 5.1 | 9.7 ± 0.2 | 0.020 – HD |
| *M. dregeanus* | 60.3 ± 3.7 | 11.5 ± 0.4 | nd |
| *M. elongatus* | 41.9 ± 2.9 | 13.1 ± 0.4 | nd |
| *M. gariepinus* | 14.6 ± 1.8 | 12.7 ± 0.5 | nd |
| *M. major* | 81.0 ± 7.2 | 15.9 ± 5.0 | HD |
| *M. pectinatus* | 45.0 ± 4.1 | 13.5 ± 0.2 | nd |
| *M. villosus* | 60.0 ± 4.3 | 11.6 ± 0.1 | nd |
| *Deplanchea tetraphylla* | 280[5] | nd | HD |
| *Dombeya a.* ssp. *acutangula* | 3.7 ± 1.5 | 18 – >50 | nd |
| *D. angulata* ssp. *rosea* | 8.5 (2–21) | 7–11 | nd |
| *Trochetia blackburniana* | 30.9 (5–72) | 22.6 (10–42) | 0.030 – HD |
| *T. boutoniana* | 49.3 (14–78) | 30.2 (13–48) | 0.036 – HD |
| *T. granulata* | 46.7 (17–101) | 13.3 (8.5–23) | HD |
| *T. triflora* | 35.8 (15–63) | 15.3 (10–19) | 0.17 – HR |
| *T. uniflora* | 61.1 (9–186) | 21.6 (15–28) | 0.027 – HD |
| *Lumnitzera littorea* | 24–52 | nd | nd |
| *Puya alpestris* | 410 | 8.2 | 0.05–0.09 – HD |
| *Banksia leptophylla* | nd | 22.0 ± 3.4 | 0.35 – HR |
| *B. sphaerocarpa* | nd | nd | 1.10 – SD |
| *B. telmatiaea* | nd | nd | 1.19 – SD |
| *Grevillea robusta* | 19.1 ± 1.0 (9–37) | 23.5 ± 1.5 (7–49) | 0.11 – HR |
| *Jaltomata biflora* | 0–47[6] | 14–58 | nd |
| *J. paneroi* | nd | nd | HD |

[1] Measurements given as means ± S.D./S.E., or means with range in parentheses, or as a range.
[2] Classes of sugar composition as defined by Baker & Baker (1983): hexose–dominant, HD (S/H ratio < 0.1); hexose–rich, HR (0.1 > S/H ratio < 0.5); sucrose–rich, SR (0.5 < S/H ratio < 1.0); sucrose–dominant, SD (S/H ratio > 1.0).
[3] Range of mean values.
[4] Production per hour per flower.
[5] Indirect measure; the volume of water a 'nectar–lobe' will hold.
[6] Depending on sexual phase.

(*b*) *Asparagales – Hemerocallidaceae* – Phormium

*Phormium* is an endemic New Zealand genus with only two species. *Phormium tenax* grows widespread throughout New Zealand (P. B. Heenan, personal communication). The flowers are orange to dark red, large and tubular, and are borne in clusters on small sub-inflorescences along a main inflorescence stalk that can reach several meters in height. Nectar is produced in large quantities (Table 2), often forming visible drops at the mouth of the corolla tube (J. L. Craig, personal communication). Nothing has been mentioned about *P. tenax* nectar colour in the literature, but yellow colouration of the nectar has been observed in plants in the native range in New Zealand (J. L. Craig, personal communication), and in plants in a botanical garden in South Africa (J. Henning, pers. comm). However, colouration of the nectar in *P. tenax* may be an exception, rather than the rule, as other researchers have only observed clear nectar (P. B. Heenan, personal communication). Nectar standing crop is higher in male-phase than in female-phase flowers (Craig & Stewart, 1988). Hence, any colouration in the nectar may be related to flower age; i.e. it may be a feature of nectar concentration by evaporation, perhaps combined with oxidative processes. The sister species, *P. cookianum*, has only been observed to have clear nectar in the field (P. B. Heenan, personal communication). The main pollinators of *P. tenax* are birds; specialised honeyeaters as well as more generalised nectarivorous species (Craig & Stewart, 1988), but two endemic *Hoplodactylus* gecko species have also been observed visiting the flowers in large numbers (Whitaker, 1987; Towns, 2002). *Phormium tenax* is a predominantly outcrossing species (Craig & Stewart,

1988), although the realised mating system is strongly influenced by local, inflorescence-level competition between selfed and outcrossed flowers (Becerra & Lloyd, 1992).

### (*c*) *Asterales* – *Campanulaceae* – Nesocodon

*Nesocodon mauritianus* is a critically endangered Mauritian plant in a monotypic endemic genus, and is only found on vertical cliffs in five small populations. The four populations in the central and northern mountains are very small, with only a few plants each (J.-C. Sevathian, personal communication; V. Florens, personal communication). The largest known population, with some 110–130 plants, is found around the waterfall Cascade Cinq Cents Pieds in the south. The plants are small shrubs, growing on ledges and in crevices in the cliff. The flowers are large and borne singly (Fig. 2A). They last for as long as 10–11 days, with male phase lasting 6–7 days and female phase 3–5 days (J. M. Olesen, unpublished data from greenhouse plants). The red nectar has a pH as high as 9.2, and the red colour turns yellow at pH < 7. The red pigment is an aurone and the yellow pigment is a flavone or a 3-glucolysed flavonol, all of which are products of flavonoid biosynthesis. The only frequent flower visitor is an introduced bird, the red-whiskered bulbul, *Pycnonotus jocosus* (Olesen *et al.*, 1998). However, the area around Cascade Cinq Cents Pieds and the nearby Alexandra Falls was until the 1980s a stronghold of a critically endangered endemic bird, the Mauritius olive white-eye, *Zosterops chloronothos* (Cheke, 1987b). This bird would be a likely pollinator, as it is the most specialised nectarivore in the genus and it visits many other Mauritian plants for nectar (Gill, 1971; Safford, 1991; Hansen, Olesen & Jones, 2002). Furthermore, the endemic diurnal gecko, *Phelsuma ornata*, is common on the mountain slopes of the central and northern *N. mauritianus* populations. Mauritian *Phelsuma* geckos are known to be regular flower visitors and pollen vectors of many plant species (Nyhagen *et al.*, 2001; Olesen, Eskildsen & Venkatasamy, 2002; Hansen *et al.*, in press; C. N. Kaiser, personal communication); *P. ornata* is likely to play a role in the pollination of the northern *N. mauritianus* populations, but no studies have been made in these populations yet. Interestingly, recent studies have found that *P. ornata* geckos show a strong preference for coloured over clear nectar in artificial flowers (Hansen, Beer & Müller, 2006), and that *P. cepediana* geckos are efficient pollinators of another, unrelated Mauritian endemic plant with coloured nectar, *Trochetia blackburniana* (Malvaceae) (Hansen *et al.*, in press).

### (*d*) *Caryophyllales* – *Caryophyllaceae* – Schiedea

*Schiedea* is a genus in the endemic Hawaiian subfamily Alsinoideae, comprising 34 species, four of which have black nectar (Table 1). These four species are divided into sections *Alsinidendron* and *Nothoschiedea*, which together are nested monophyletically within *Schiedea* (Wagner, Weller & Sakai, 2005). *Schiedea* is one of the most remarkable examples of adaptive radiation among Hawaiian angiosperms, with a large number of evolutionary shifts in morphology, breeding systems, ecological adaptations and pollination biology (Weller & Sakai, 1990; Weller *et al.*, 1990; 1998). The four species with black nectar are found on two different islands in the Hawaiian archipelago (Table 1). The Kaua'i species are herbaceous vines, while the O'ahu species are woody shrubs and thus probably longer lived. In the greenhouse, the Kaua'i species live longer than one year, so they are not strict annuals (S. G. Weller, personal communication). The flowers are apetalous but have white or pale green petaloid sepals (Fig. 2H). Older taxonomical treatments of these species do not mention the black nectar (e.g. Mann, 1866; Sherff, 1944). The first published evidence of the coloured nectar is found in Weller & Sakai (1990), in which a colour photograph of *S. lychnoides* shows a large drop of black nectar on the tip of a petaloid sepal. Later, Weller, Sakai & Wagner (1995) reported that "nectar appears black when large amounts accumulate". In a new taxonomical revision of the genus the black nectar is finally mentioned in the description of the four species (Wagner *et al.*, 2005). The black nectar in *Schiedea* may be related to ornithophily (Weller *et al.*, 1998; Wagner *et al.*, 2005), but there are no observations of birds visiting the flowers. This is most likely due to the rarity of the plants in the wild, and the fact that much of the Hawaiian nectarivorous avifauna has gone extinct (Wagner *et al.*, 2005; S. G. Weller, personal communication).

### (*e*) *Ericales* – *Marcgraviaceae* – Schwartzia

*Schwartzia* is a neotropical genus of shrubs with 14 species in the monophyletic subfamily Noranteoideae, which comprises four small genera. While morphologically well supported, recent molecular work suggests that the four genera may not be well delimited within Noranteoideae (de Roon & Dressler, 1997; Ward & Price, 2002; Dressler, 2004). *Schwartzia brasiliensis* is a scrambling, partly epiphytic shrub from Brazil. The branches of *S. brasiliensis* carry long, terminal, brush-like racemes of up to 40 cm in length, with 60–300 flowers, each of which has the cup-like nectary connected to the base of the peduncle (Fig. 2F) (Sazima, Buzato & Sazima, 1993; Dressler, 2004). The strikingly blue-coloured nectar in *S. brasiliensis* was reported by Sazima *et al.* (1993) and Pinheiro *et al.* (1995), and is produced in vast quantities in each nectary (Table 2). A nectary can hold 100–150 μL and produce 50 μL of nectar within a 3-hour period, and drops of overflowing nectar can often be observed on the nectaries (Fig. 2F) (Sazima *et al.*, 1993; Pinheiro *et al.*, 1995). The pigment causing the blue colouration of the nectar is an anthocyanin (Sazima *et al.*, 1993). The ruby-red long stalks and flowers, and the dark purple-brown nectaries, contrast well against green foliage (Sazima *et al.*, 1993), and, in turn, the blue nectar is easily visible against the dark nectaries (Fig. 2F). *Schwartzia brasiliensis* is visited and pollinated by a wide range of birds, including both hummingbirds and perching passerines (Sazima *et al.*, 1993; Pinheiro *et al.*, 1995), and even woodpeckers (Rocca *et al.*, in press). Based on foraging behaviour, Sazima *et al.* (1993) suggested that perching passerine birds were more likely to be efficient pollinators, than hovering hummingbirds. During night, when the flowers have already wilted, nocturnal insects and perhaps

bats forage on the remaining nectar, which in old nectaries can smell of cabbage (Sazima *et al.*, 1993).

(*f*) *Fabales – Fabaceae*

(*i*) *Calliandra*. This is a large, widespread tropical genus with about 200 species (Mabberley, 1997). Yellow nectar has been observed in *C. calothyrsus* over much of the species' native range (J. R. Chamberlain, personal communication), and also in naturalised and plantation trees in Western Kenya (Jensen, 2005) and the Philippines, where honey bees produce a green honey based on the yellow nectar (Estolas, 2004). The native range of *C. calothyrsus* stretches from southern Mexico to central Panama (Chamberlain, 1998), but the species is also widely used in many tropical countries for agroforestry, fodder and honey production (Palmer, Macqueen & Gutteridge, 1994; Chamberlain, 2000). The mimosoid flowers are small and green and are borne in large umbelliform clusters, but each flower has numerous red staminal filaments of 4–6 cm in length. Many different flower visitors have been observed in the native range of *C. calothyrsus*: bats, birds, large hawkmoths, honey bees and other insects. However, the nocturnal short anthesis of individual flowers means that the only efficient pollinators are bats and large hawkmoths (Palmer *et al.*, 1994; Chamberlain, 2000). If the plentiful nectar is left unharvested overnight, birds will feed on it in the morning until the flowers wilt and drop off, but by then the stigma is no longer receptive. Non-native honey bees and other insects also visit the flowers in the morning, but they all approach the nectar at the bottom of the flowers and never touch the fertile parts, and are thus unlikely pollinators (Chamberlain, 2000; Jensen, 2005).

(*ii*) *Erythrina*. A genus with 112 species, found both in paleotropical and neotropical regions (Bruneau, 1996; Mabberley, 1997). Coloured nectar was reported by Guillarmod, Jubb & Skead (1979) in three species of the two closely related small sections *Humeanae* and *Caffrae* (Krukoff & Barneby, 1974; Bruneau, 1996; 1997), but nothing more is known about coloured nectar in *Erythrina*. The flowers are usually grouped in large inflorescences, and are strongly zygomorphic, with most of the inner petals usually being enclosed by the large outer standard. Thus, the nectar is rarely visible from outside, and the relatively weak colours of the nectar are probably not visible against the bright red corollas (Table 1). The nectar colours reported by Guillarmod *et al.* (1979) may be due to local edaphic (e.g. pH or nutrient levels) or climatic factors in the areas where they investigated the plants, as other researchers have failed to notice any significant colouration of the nectar in these species (S. W. Nicolson, personal communication; F. Forest, personal communication). The genus is believed to be entirely ornithophilous, and flowers of most species produce large quantities of nectar. However, insects, lizards and mammals have also been observed visiting flowers, but mostly in very low numbers (Scott-Elliot, 1890; Raven, 1974; Toledo, 1974; Bruneau, 1997; Sazima, Sazima & Sazima, 2005). Guillarmod *et al.* (1979) report that the three species with coloured nectar are visited by a wide range of nectar-feeding bird species.

(*g*) *Gentianales – Apocynaceae* – Hoya

*Hoya* is a large, tropical genus with 200–300 currently recognised species. It is found throughout most of the Indo-Australian region. The centres of diversity are found in the Indonesian archipelago, with approximately 25 species in Malaysia, 74 in New Guinea and >30 species in the Philippines (Forster, Liddle & Liddle, 1998; Kleijn & van Donkelaar, 2001; Wanntorp, Kocyan & Renner, 2006; Wanntorp *et al.*, in press). *Hoya* belongs to the subfamily Asclepiadoideae, which has the most elaborate and complex floral structure of all eudicots (Endress, 1994). Most *Hoya* species grow as vines or arboreal shrubs, with flowers arranged in umbels. Apart from information on their habitat, almost nothing is known about their ecology. However, many species are popular as ornamental plants (waxflowers, porcelain flowers), and it is from dedicated hobby plant growers that we received much of the information on coloured nectar in this genus. Many *Hoya* species produce large amounts of nectar, which forms visible drops on the flat corollas of the flowers, including the ones with coloured nectar (Fig. 2B,C). Coloured nectar is only found in the five species of section *Amblyostemma* (Table 1), and Kloppenburg (1994) included "exuding a coloured honeydew" in his list of diagnostic characters (synapomorphies; specialised characters shared by two or more species, originating in their last common ancestor) for the section. Nothing is known about the nectar properties or pollination biology of any of the species in this section. The only study of pollination biology in *Hoya* is from Australia, in which Forster (1992) found *H. australis* to be pollinated by Lepidoptera. In fact, the asclepiads were thought to be strictly entomophilous (Ollerton & Liede, 1997), until Pauw (1998) elegantly demonstrated tongue-pollination by sunbirds in one species.

(*h*) *Geraniales – Melianthaceae* – Melianthus

*Melianthus* is a small South African genus with eight species in what has been called an enigmatic Southern African family, whose taxonomy and floral morphology have long puzzled botanists (Ronse Decraene *et al.*, 2001). The petals are small, fused and red or pinkish, and are mostly hidden in a cup formed by the large sepals. However, in some of the species, namely *M. elongatus*, *M. pectinatus* and *M. gariepinus*, the petals are large and showy enough to be considered as the main visual attractant of the flowers (J. Henning, personal communication). In some of the species, the large drop of dark nectar can be seen not only from the front of the flowers, but also from the sides because the sepals are pale, thin and translucent (Fig. 1). The dark nectar in *Melianthus* has been known at least since 1795 (see Section I), and has received anecdotal references in publications since then (e.g. Scott-Elliot, 1890; Dyer, 1952; Archer & Condy, 1997). It is unclear to what extent *Melianthus* species vary in nectar colour within and between species; whereas *M. comosus*, *M. elongatus* and *M. villosus* have all been

observed to have black nectar, most of the remaining species have brown nectar (J. Henning, personal communication). No thorough pollination study has been carried out, but both insects and birds have been observed visiting *Melianthus* species for nectar. Scott-Elliot (1890) reported that the "abundance of rich black honey which sometimes almost fills the cup" was eagerly sought by sunbirds, and Marloth (1925) remarked that "the nectar of *M. comosus* is so dark that the honey taken from the hives of bees which feed on it is quite black". Mabberley (1997) also includes, in his characteristic shorthand, the intriguing comment "fls rich in nectar (black, taken by sunbirds)".

### (*i*) *Lamiales – Bignoniaceae*

(*i*) *Deplanchea*. A genus with five species, ranging from Sumatra to New Caledonia. *Deplanchea tetraphylla* is a large tree, found in New Guinea, the Aru Islands, and North East Queensland, Australia (Avé, 1984). It is apparently the only species in the genus with coloured nectar, which it presents in a most spectacular and specialised manner. Its large yellow flowers are clustered in large, circular and flat inflorescences at the end of upper branches. Each flower is strongly zygomorphic with a lower central corolla lobe forming a spoon-like hollow where the dark-brown nectar accumulates (Fig. 2N; Weber & Vogel, 1986). Known flower visitors include birds (Brown & Hopkins, 1995) and mammals (Jackson, 2001). Weber & Vogel (1986) concluded that birds were most likely to be the main pollinators throughout most of the range of *D. tetraphylla*. Furthermore, they stated that the easy, open access to the nectar is suggestive of pollination by relatively short-beaked, generalised nectar-feeding birds, such as lorikeets. In Australia, birds and large butterflies visit the flowers in great numbers (G. Sankowsky, personal communication). Weber & Vogel (1986) suggested that the brown pigment in the nectar was a melanin. Furthermore, they remarked that in New Guinea, younger flowers were seen with honey-coloured nectar, while older flowers had darker brown nectar.

(*ii*) *Fernandoa*. A genus with 14 species with a paleotropical distribution stretching from Africa and Madagascar to China, South-east Asia and Sumatra (Mabberley, 1997). *Fernandoa magnifica* is a large central-eastern African tree. It often flowers while leafless; inflorescences consist of cymes of 4–10 upwards-turned, large campanulate flowers (Dale & Greenway, 1961). The flowers are red at the top, grading through orange to yellow at the base, where copious amounts of dark brown, almost black nectar forms a small pool (Fig. 2O). Pollinators are unknown, but the flowers have an unpleasant, cabbage-like smell (C. Puff, personal communication, M. Hyde; personal communication), which may be indicative of bat-pollination (Knudsen & Tollsten, 1995). However, the large, colourful flowers also suggest that birds may be attracted to the flowers and act as pollinators, and there are plant species that are pollinated by both bats and birds (Sazima, Sazima & Buzato, 1994; Muchhala, 2003). Almost nothing is known about nectar colour in the rest of the genus, but the Malagasy *F. madagascariensis* has 6–7 cm large ornithophilous flowers with small amounts of clear nectar (J. M. Olesen, personal observations).

### (*j*) *Lamiales – Lamiaceae* – Leucosceptrum

*Leucosceptrum* is a very small genus with three species, found from the Himalayas to China (Mabberley, 1997). *Leucosceptrum canum* is a common species, with small, cream-white flowers arranged in dense, terminal spikes. Nothing is noted about its dark brown nectar in the literature, the only information is from an observation in a commercial nursery in the Indian Himalayas (G. M. Pradhan, personal communication). The species is known as a honey plant (Li & Hedge, 1994), and Cowan & Cowan (1929) remarked that, "a quantity of sweet juice exudes from the flowers, and this is sucked by Paharia herdsmen, and by many birds". The former are probably less efficient than the latter as pollinators, though, and it can therefore be assumed that the plant is ornithophilous. A study on the species in China (Caihuan *et al.*, 2004) identified a yellow pigment from the flowers as an amine, but it is not clear whether the pigment analysed was from the floral parts or the nectar.

### (*k*) *Malvales – Malvaceae*

(*i*) *Dombeya*. A large, widespread genus with about 225 recognised species in Africa, Madagascar and some of the Indian Ocean islands (Mabberley, 1997). The evolutionary hotspot and likely origin of *Dombeya* is in Madagascar (including the Comoros Islands) with approximately 190 species. The Ethiopian *D. kefaensis*, the Malagasy *D. cacuminum* and the Mascarene *D. elegans*, *D. acutangula* ssp. *acutangula*, *D. acutangula* ssp. *palmata* and *D. acutangula* ssp. *rosea* are the only known *Dombeya* with coloured nectar. In the taxonomical description of *D. kefaensis*, Friis & Bidgood (1998) mention a dark orange nectar stain at the base of each petal, and in their Fig. 2 the dark red nectar is clearly seen, contrasting with the pale cream petals. Malagasy *D. cacuminum* plants in cultivation elsewhere have been reported to produce abundant musky-scented yellow nectar (Llamas, 2003). Another rare floral trait, secondary pollen presentation on the tip of the petals, has been reported for this species (Prenner, 2002). Together, these two floral traits could suggest a combination of bird- and bat pollination for *D. cacuminum* (Llamas, 2003). In a revision of continental African *Dombeya* species, Seyani (1991) lamented the fact that virtually nothing is known about their pollination biology. However, there have been some studies of the reproductive biology of *Dombeya* spp. on the island of La Réunion (Gigord, Lavigne & Shykoff, 1998; Gigord, Picot & Shykoff, 1999; Humeau & Thompson, 2001 and references therein), with a few of those studies providing anecdotal observations on flower visitors. In two studies of *D. acutangula* ssp. *acutangula*, Gigord *et al.* (1998, 1999) mention insects and birds visiting the flowers that produce large quantities of nectar. There is no reference to the yellow colour of the nectar in any of the studies mentioned above, but it is always strikingly yellow when present (L. Gigord, personal communication). The majority of *Dombeya* species have flowers with either white or pinkish corollas, and many have large, showy inflorescences with rather small flowers. The small, white-flowered species are probably pollinated mainly by insects, whereas

larger, pinkish and red ones such as *D. lastii* may be bird pollinated (Seyani, 1991). Indeed, *D. goetzenii* with pale pink flowers that are deep red at the base of the staminal tube (Dale & Greenway, 1961) is visited by several sunbird species (Cheke & Mann, 2001).

(*ii*) *Trochetia*. An endemic Mascarene genus encompassing six species of shrubs and small trees; five species in Mauritius and one in La Réunion. All six species have coloured nectar, and show a remarkable variation in flower morphology and colour among species (Fig. 2G; Table 1; Friedmann, 1987). Their proposed closest relatives include several Malagasy Dombeyoid genera (Friedmann, 1987). The most commonly reported pollinators of the Mauritian *Trochetia* species are two nectarivorous endemic bird species: the Mauritius grey white-eye, *Zosterops mauritianus*, has been observed visiting *T. blackburniana*, and the Mauritius olive white-eye, *Z. chloronothos*, has been observed on *T. uniflora* and has repeatedly been suggested as the main pollinator of *T. blackburniana* (Gill, 1971; Staub, 1988; Safford, 1991; Hansen *et al.*, 2002). Furthermore, *Z. chloronothos* has been suggested as a pollinator of the endangered *T. boutoniana* (Staub, 1988). In La Réunion, both endemic species of *Zosterops* have been observed visiting *T. granulata* (Gill, 1971; D. M. Hansen, personal observations). An anecdotal observation of an endemic diurnal gecko visiting a flower of *T. blackburniana* (Staub, 1988) has recently been confirmed by a study which shows that Mauritian *Phelsuma* geckos are important pollinators of *T. blackburniana* (Fig. 2Q; Hansen *et al.*, in press). *Phelsuma* geckos have been confirmed to visit a wide range of other Mauritian endemic plant species (Nyhagen *et al.*, 2001; Olesen *et al.*, 2002; D. M. Hansen, personal observations; C. N. Kaiser, personal communication), and in a recent study *P. ornata* geckos strongly preferred coloured over clear nectar in experimental artificial flowers (Hansen *et al.*, 2006). More studies on how *Phelsuma* geckos interact with Mauritian plants with coloured nectar in the wild are needed to assess the effect of coloured nectar on reproductive success. *Trochetia blackburniana* may be well suited for such studies, as its nectar naturally varies from clear to deep yellow or orange, even within small populations, while variation in nectar colour of flowers on the same plant seems to be smaller (D. M. Hansen, personal observations).

*Dombeya* and *Trochetia* both belong to the subfamily Dombeyoideae. It seems curious that the occurrence of coloured nectar within Dombeyoideae in Madagascar, the evolutionary hotspot of the subfamily, should be restricted to *D. cacuminum*. It is very likely that further field studies will reveal coloured nectar in more of the Malagasy Dombeyoid genera or species. Given the wide distribution of many *Dombeya* species in Africa (Seyani, 1991), it is also likely that coloured nectar will be found in additional African species.

(*l*) *Myrtales – Combretaceae* – Lumnitzera

*Lumnitzera* is a genus with only two species, both of which are widespread in mangrove forests from East Africa to the Western Pacific and tropical Australia (Tomlinson, 1986). *Lumnitzera littorea* has terminal inflorescences with small, red flowers, exerted stamens and a cup formed by the calyx, allowing for abundant nectar to accumulate (Table 2). The flowers thus fit well into the general ornithophilous syndrome, and are predominantly visited by sunbirds and honeyeaters, but also by bees and wasps (Tomlinson, 1986). Yellow nectar in *L. littorea* has only been reported from Guam (H. Skovsgaard, personal communication), but nothing is known about nectar colour anywhere else in this species' wide range. However, the nectar of plants of the sister species, *L. racemosa*, growing in the Fairchild Botanical Garden, Florida, USA, was observed to be clear (P. B. Tomlinson, personal communication).

(*m*) *Poales – Bromeliaceae* – Puya

*Puya* is a terrestrial bromelioid genus with almost 200 species that are mainly found on the slopes of the Andes Mountains from Chile to Columbia (Smith & Downs, 1974). Recent molecular taxonomical work assigned *Puya* as a monophyletic sister genus to the subfamily Bromelioideae (Terry, Brown & Olmstead, 1997; Givnish *et al.*, 2004). The subgenus *Puya* comprises eight species with large, terminal, columnar, and multibranched inflorescences (Hornung-Leoni & Sosa, 2004); the most well known being the Peruvian *P. raimondii* – the 'Queen of the Andes' – which grows to 10–12 m in total height (Sgorbati *et al.*, 2004). *Puya alpestris* is confined to Chile, and is smaller, with an inflorescence of 1–1.5 m and up to 2 m total height (Hornung-Leoni & Sosa, 2006). Each branch on the inflorescence carries several large flowers of a striking blue colour with bright orange pollen. Nectar is produced in vast quantities, and sometimes it drips out of the flowers. In the wild, the nectar has been observed to be varying in colour from pale pink to a red-wine like colouration (C. Hornung-Leoni, personal communication). In plants grown in gardens in California and in the UK it has been reported to be of a blue colour (Dortort, 2003; D. Poole, personal communication). In all species of subgenus *Puya*, the terminal half of each branch is sterile, with only bracts and no flowers. This was proposed by Johow (1898) to be an adaptation to pollination by perching nectar-feeding birds, which are the main pollinators of the subgenus – as opposed to the subgenus *Puyopsis*, which is mainly hummingbird-pollinated and lacks such perches. This dichotomy is reflected in the nectar composition as well: nectars from subgenus *Puyopsis* are sucrose-rich and of relatively high concentration, while nectars from subgenus *Puya* are relatively dilute and hexose-rich (Table 2; Scogin & Freeman, 1984; Baker & Baker, 1990). Observed pollinators of *P. alpestris* include the Austral blackbird, *Curaeus curaeus*, and *Castnia eudesmia*, a large diurnal moth that also passes its larval and pupal stages on *Puya alpestris*, and which is known occasionally to drive off other moths and birds (!) from the flowers (Gourlay, 1950; Ortiz-Crespo, 1973). Nothing is known about what causes the blue colouration of the nectar, but an unusual floral anthocyanin, delphinidin 3,7,3'-triglucoside, has been identified as the main petal colour pigment (Scogin & Freeman, 1984; Scogin, 1985), and it could be related to the nectar colouration as well. Coloured

nectar is not known from any other Bromeliaceae, and a recent study of nectar from 110 bromeliad species from 16 genera, including five *Puya* species, found all of these to be clear (T. Krömer, unpublished data).

(*n*) *Proteales – Proteaceae*

(*i*) *Banksia*. An Australian genus with 73 species (Mabberley, 1997). Five *Banksia* species from the *Sphaerocarpa* group (series *Abietinae*) were reported to have an initially yellow nectar, which turns olive to dark green, almost black, and gelatinous during anthesis (Lamont, 1980; Markey & Lamont, 1995). The dark nectar is easily seen deep in the inflorescence, between the long filaments (Fig. 1 in Lamont, 1980). The nectar of another species, *B. nutans*, remains a translucent yellow throughout anthesis. At first this was assumed to be a secondary effect of a presence of cyanobacteria, but a subsequent analysis rejected this hypothesis (Markey & Lamont, 1995). What causes the colouration of the nectar is still unknown (B. B. Lamont, personal communication). However, it is likely to be a chemical process in the nectar itself, as a colour change also occurs in freshly secreted yellow nectar after storage in sterile tubes (Markey & Lamont, 1995). The inflorescences of all *Banksia* species in series *Abietinae* are spherical, relatively close to the ground, and produce large volumes of nectar. Observed pollinators and flower visitors include honeyeaters, ants, flies and beetles. The strong, musky smell of the flowers of these species also suggests nocturnal mammals, and scats have often been found on inflorescences, lending further credibility to this hypothesis (Markey & Lamont, 1995; B. B. Lamont, personal communication). Indeed, several non-flying mammals, e.g. the honey possum, are efficient pollinators of Australian plants, including several *Banksia* species (Carthew & Goldingay, 1997; Wooller & Wooller, 2003).

(*ii*) *Grevillea*. A large Australasian genus with 261 species, many of which are ornithophilous (Mabberley, 1997). Ornamental *Grevillea robusta* plants in South Africa and Kenya have been reported to have yellow nectar, and red nectar has been observed in a greenhouse plant in Germany (Kalinganire *et al.*, 2001; Nicolson, 1993; 1995; J. Henning, personal communication), but curiously nobody has reported coloured nectar from its native range in Australia (R. O. Makinson, personal communication). The red colouration at the base of the petals makes the yellow nectar seem almost orange (Fig. 2I). There are many observations of nectar-feeding birds on the flowers of *G. robusta* in its native range (Makinson, 2000), and native flying foxes (*Pteropus*, Macrochiroptera) have also been observed visiting the flowers (R. O. Makinson, personal communication).

(*o*) *Solanales – Solanaceae*

(*i*) *Capsicum*. Also known as chilli-peppers, *Capsicum* is a neotropical genus with 20–27 recognised species. Chilli peppers have been cultivated in many parts of South America for 7000–8500 years, and are now grown in many parts of the world (Walsh & Hoot, 2001). The genus is closely related to *Jaltomata* (Vogel, 1998). Yellow nectar is found in species from the earliest diverging branches of the phylogeny of the genus, in the two closely related groups *Eximium* and *Baccatum*, and in the unassigned species *C. pubescens* (Walsh & Hoot, 2001). The yellow nectar is visible as well-defined drops on the cream-white corolla of *C. baccatum*. In the purple-flowered species *C. pubescens* and *C. eximium* the yellow nectar contrasts against white spots near the centre of the corolla, and is also clearly visible (Fig. 2P). Despite the current widespread cultivation of at least one of the *Capsicum* species with coloured nectar (*C. baccatum* varieties), nothing has previously been published about the yellow nectar it very often produces. The only flower visitors that have been observed on plants with coloured nectar in the field are ants (W. H. Eshbaugh, personal communication). Based on the current understanding of the phylogenetic relationships within the genus (Walsh & Hoot, 2001) it is likely that more *Capsicum* species might produce yellow nectar, namely *C. cardenasii* and *C. tovarii* (W. H. Eshbaugh, personal communication). Self-incompatibility in the genus seems to be facultative; plants typically exhibit self-incompatibility for a period, but if outcrossing does not occur, plants will 'allow' self-pollination and produce a small amount of fruit toward the end of the fruiting season (W. H. Eshbaugh, personal communication).

(*ii*) *Jaltomata*. *Jaltomata* is a diverse neotropical genus of 46 species of perennial herbs and shrubs, and is distributed from Arizona, USA, to Bolivia, the Galápagos Islands and the Greater Antilles. A subset of the species that grow in Peru and Bolivia produce orange to red nectar; all other species produce clear nectar. These species represent some of the most striking examples of coloured nectar; for example, Bitter (1924) quoted Weberbauer's vivid description of how *J. umbellata* secretes "blood red juice ... which often fills the whole tube" that can be seen through the pale green corolla – similar to red wine in a glass (Fig. 2K). *Jaltomata umbellata* and *J. aspera* grow in the Lomas formation, a fog-fed desert habitat of the west coast of South America with a high level of endemism (Dillon, 1997). The remaining *Jaltomata* species with coloured nectar grow in higher and moister habitats, at altitudes up to 3800 m (Table 1). The species with coloured nectar show marked variation in flower shape (Table 1), and have corollas that remain open at night. Among the *Jaltomata* species with clear nectar, some species have corollas that remain open at night, and others have corollas that close in the late afternoon and open again the next day. Coloured nectar tends to be produced in greater volumes than clear nectar, but this trend is not absolute. *Jaltomata paneroi*, *J. umbellata*, *J. ventricosa* and *J. weberbaueri* all produce copious red or orange nectar, whereas *J. antillana*, *J. confinis*, *J. grandiflora*, *J. procumbens*, *J. repandidentata* and *J. sinuosa* produce clear nectar in minute amounts. Quantitative nectar data are only known for one species with coloured nectar, *J. biflora* (Table 2). The nectar in *J. biflora* is usually clear but can turn amber or orange in colour as the flower ages (Mione, Mugaburu & Connolly, 2001). Nothing is known about the pollination biology of

*Jaltomata*, including the species with coloured nectar. Bitter (1921) quoted a correspondence with Weberbauer, in which he suggested that the red nectar of *J. aspera* attracts small flies for pollination. Bitter (1921) did not state whether Weberbauer had seen the flies or was speculating, but the size of flowers and the large distance from the nectar to the reproductive surfaces (Fig. 2L) make it unlikely that small flies could act as pollinators. Later, Mione & Anderson (1996) speculated that the red nectar in *Jaltomata* was related to hummingbird pollination, based on floral morphology and the red colouration common among hummingbird flowers (e.g. Proctor, Yeo & Lack, 1996).

## IV. DISCUSSION

Our review demonstrates that coloured nectar is geographically and taxonomically widespread, but also relatively rare and poorly documented. However, it is very likely that many more plant species have coloured nectar, and that it has so far been under-reported in the scientific literature. Nevertheless, the information we have presented allows us to discuss environmental and ecological patterns, in addition to the evolution of coloured nectar.

### (1) Environmental and ecological correlates of coloured nectar

The occurrence of coloured nectar seems to be correlated with three factors. Firstly, vertebrate pollination: many plants with coloured nectar are known to or have been hypothesised to be vertebrate-pollinated, mainly by birds (Table 1). Secondly, insularity: *Nesocodon mauritianus*, *Trochetia* spp., *Dombeya acutangula* ssp. *acutangula* and ssp. *rosea*, *D. cacuminum*, as well as the *Schiedea* spp. are all island endemics, while the majority of the other species are found in insular mainland areas, isolated either by altitude (e.g. most *Jaltomata* spp. and *Puya alpestris* in the Andes Mountains, *Leucosceptrum canum* in the Himalayas, and *Dombeya kefaensis* in Ethiopia), habitat (*Jaltomata umbellata* and *J. aspera* grow in the Peruvian Lomas formations – vegetation islands surrounded by hyperarid desert) or by biogeographical history (i.e. *Aloe* spp. and *Melianthus* spp. in Southern Africa, *Hoya* spp. in insular Asia, and *Banksia* spp. and *Grevillea robusta* in Australia). Thirdly, most plants with coloured nectar occur at high or relatively high altitudes (Table 1) – e.g. for a plant in Mauritius, 600–800 m is a high altitude, as the maximum height of the island is 828 m.

There are some notable exceptions to the second and third factors listed above, including *Hoya*, where some species with coloured nectar occur in mainland Asia, and all *Hoya* species with coloured nectar are lowland plants. Also, *Grevillea robusta* is found from sea level to higher altitudes, as is *Calliandra calothyrsus*, *Fernandoa magnifica*, *Deplanchea tetraphylla*, *Phormium tenax* and some of the *Melianthus* species (Table 1). Lastly, being a mangrove forest plant, *Lumnitzera littorea* is only found at sea level.

Furthermore, there may be cases where intercorrelations can explain some of the patterns. For example, bird pollination and altitude are correlated, as birds are important pollinators in many high-altitude tropical ecosystems (Cruden, 1972; Wolf & Gill, 1986; Kessler & Krömer, 2000). Similarly, it is likely that there is an intercorrelation between insularity and vertebrate pollination. Bird- and non-bird vertebrate pollination may be more prevalent on islands than in comparable mainland areas, due to a depauperate invertebrate pollinator fauna on islands (Elmqvist *et al.*, 1992; Feinsinger, Wolfe & Swarm, 1982; Olesen, 2003; Olesen & Valido, 2003).

### (2) Coloured nectar and vertebrate pollination

One straightforward interpretation of coloured nectar across all taxa is to see it as a visual floral cue to potential pollinators. In this study, we often find coloured nectar to be associated with observed or hypothesised ornithophily. Hence, it is tempting to interpret this floral trait as another example of visual floral adaptation to ornithophily. However, whether or not different floral traits fit neatly into clearly defined pollination syndromes has recently been the subject of debate, and some researchers have argued that syndromes are not very useful predictors of pollinators (reviewed by Fenster *et al.*, 2004). Nevertheless, there are many studies that find syndromes to be valid and useful, at least to predict flower visitors or functional groups of most efficient pollinators at higher taxonomic levels (e.g. Bruneau, 1997; Lange, Scobell & Scott, 2000; Temeles & Kress, 2003; Hargreaves, Johnson & Nol, 2004; Wilson *et al.*, 2004). Fenster *et al.* (2004) conclude that pollination syndromes are of great use in understanding the ecology and evolution of plant-pollinator interactions, and suggest that pollinators can be organised into functional groups according to the selection pressures they exert. Since many taxa with coloured nectar are visited by birds, or have been hypothesised to be visited by birds (Table 1), we can ask the question: do the observed nectar colours correspond to typical bird-colours of flowers? For yellow and especially orange and red, the answer is yes (e.g. Proctor *et al.*, 1996). For darker nectar colours, especially black, the answer may also be yes if we substitute flower colour with fruit colour, as black is the most frequent fruit colour among bird-dispersed plant species in the tropics (Herrera, 2002; van der Pijl, 1982; Wheelwright & Janson, 1985; Willson & Whelan, 1990).

Furthermore, where known, most coloured nectars are hexose-dominated (Table 2), which could be an adaptation to generalised bird pollinators in habitats with a general dearth of pollinators, such as islands and montane areas (Cruden, 1972; Wolf & Gill, 1986; Barrett, 1996; Olesen & Jordano, 2002; Anderson, 2003). Generalised nectar-feeding birds often lack the specific enzymatic activity required to digest sucrose, and can thus only utilise the simpler hexose sugars (e.g. Martínez del Rio, Baker & Baker, 1992).

Therefore, it is conceivable that several different generalised avian pollinators in insular or montane habitats can

exert a relatively uniform selective pressure on one or more floral traits. For example, in the Canary Islands many unrelated plant lineages show a remarkable evolutionary flexibility in relation to nectar sugar composition. It has been hypothesised that this could be caused by selective pressures exerted by several generalist nectar-feeding passerine birds (Dupont *et al.*, 2004).

However, birds are not the only vertebrates associated with coloured nectar. In Mauritius, at least one and possibly two or three *Trochetia* species are pollinated by endemic diurnal *Phelsuma cepediana* geckos (Hansen *et al.*, in press), and two of the three *Nesocodon mauritianus* populations are found on cliffs, which harbour *Phelsuma ornata* geckos that are also well-known flower visitors (Nyhagen *et al.*, 2001; Olesen *et al.*, 2002), and which react strongly to coloured nectar as a visual signal for reward (Hansen *et al.*, 2006). Furthermore, *Phormium tenax* in New Zealand is visited by endemic geckos, albeit nocturnal species. A recent review demonstrated that lizard pollination was almost exclusively an island phenomenon (Olesen & Valido, 2003). Hence, further studies may reveal even more lizard pollinators for island plants with coloured nectar. Lastly, some coloured nectar plants are also visited and pollinated by nocturnal mammals (Table 1; *Deplanchea tetraphylla*, *Fernandoa magnifica*, *Calliandra calothyrsus*, *Dombeya cacuminum* and *Banksia* spp. – and *Trochetia triflora* may have been pollinated by one of the two extinct small Mauritian fruitbat species, *Pteropus subniger* and *P. rodricensis*). While it is more difficult to envisage a visual function of coloured nectar during night, it may be related to diurnal pollinators for some of these species (*D. tetraphylla*, *F. magnifica* and *Banksia* spp.), or it may serve functions other than pollinator attraction or be a non-functional trait (see below).

### (3) Coloured nectar and insect pollination

Obviously, coloured nectar could also function as a visual cue to flower visiting or pollinating insects. Small, insect-pollinated flowers are probably even more unlikely to have had any colouration of their nectar documented than the relatively large flowers displayed by most of the plant species in our review. This could explain the bias towards large, vertebrate-pollinated flowers in our current knowledge of coloured nectar. We have a very incomplete knowledge of flower visitors for many of the plant species with coloured nectar, and some of the plants are likely to be mainly insect-pollinated (*Hoya* spp. and *Capsicum* spp.). These species are thus ideal candidates for studies investigating the potential functional significance of coloured nectar for insect pollinators or flower visitors.

### (4) Coloured nectar as an honest signal

Thorp *et al.* (1975) suggested that the UV-reflectance of some nectars could be interpreted as a visual cue for flower-visiting insects, especially bees, allowing them to evaluate the presence and perhaps the abundance of nectar. Similarly, Weber & Vogel (1986) suggested a functional significance of coloured nectar in the human visible spectrum. They interpreted the dark nectar against the yellow corolla in *Deplanchea tetraphylla* as a visual signal, a nectar guide, in addition to its primary function as a reward. Olesen *et al.* (1998) also suggested this link between signal and reward, and having observed birds visiting *Nesocodon mauritianus*, they speculated further that the coloured nectar could be interpreted not only as a visual cue, but additionally as an honest signal, leading to increased pollination efficiency (the idea was originally suggested to J. M. Olesen by W. S. Armbruster). However, they rejected this possibility, since – to their knowledge – coloured nectar had not evolved in other regions and taxa. With our review we demonstrate that the opposite is true: coloured nectar has evolved independently and repeatedly in many geographical regions and in many plant taxa. This pattern of convergence suggests the possibility of a common selective pressure, such as the need for an honest signal (*sensu* Zahavi, 1975, 1977) to flower visitors, allowing them to judge the presence and size of the reward before flower visitation, and to adjust their behaviour accordingly.

Recently, another little-studied nectar trait, scent, was proposed to be a potential honest signal (Raguso, 2004b). He argued that scented nectar could facilitate remote detection of the nectar by potential pollinators, as well as providing an assessment of nectar quantity in individual flowers. A similar argument has been put forward in relation to the potential function of pollen odours by Dobson & Bergström (2000), but they did not discuss the potential for honest signalling here. Scent may not always be a precise signal, as it strongly depends on environmental conditions. However, compared to visual signals that rely on an unobstructed straight line between sender and receiver, scent signals could be more efficient, as these can also be percieved without a straight line between sender and receiver. Additionally, some floral oils are known to be coloured (Buchmann & Buchmann, 1981; Buchmann, 1987), and this could act as a visual honest signal in a parallel way to coloured nectar. Lastly, being a harvestable floral resource like nectar, pollen has also been shown to function as a visual signal (reviewed in Lunau, 2000), but unlike nectar, pollen in individual anthers is not a renewable floral resource. Once an anther has dehisced, it can produce no more pollen. However, temporal patterns of anther dehiscence during anthesis of individual flowers or inflorescences do provide a plant with some control over the amount of pollen available for harvest (Lunau, 2000). Hence, in some plant species, pollen may well function as an honest signal, too.

In general, for a signal to be honest, it has to infer some cost, a 'handicap', upon the signaller (Grafen, 1990; Zahavi, 1975; 1977). In coloured nectar plants, the cost can be at least twofold: firstly, colour compounds may be costly to produce. Here, the cost might be directly related to the production of the signal. However, until we have more knowledge on which processes or substances cause colouration of nectar, the biochemical cost of producing coloured nectar is impossible to calculate. Secondly, the cost can be seen as the number of visits that a flower does not receive when the nectar has been depleted and the signal is absent. In this scenario, contrary to conventional honest signalling theory, it is not the production or

presence of a signal that is costly; it is rather the absence of the signal that is costly, as the flowers will not receive further visits until the signal has been replenished. Because signal and reward are coupled, an additional cost of having coloured nectar could be the loss of the plant's ability do deceive its pollinators and manipulate their movement patterns.

The benefit of coloured nectar to the pollinator is clear: it directs foraging towards rewarding flowers and promotes avoidance of visits to empty flowers, thus reducing foraging costs. The benefit to the plant is more complex. At first glance, coloured nectar could seem to be disadvantageous to the plants; penalising them because they receive fewer visits when the nectar is missing. However, as mentioned above, signal and reward are coupled in coloured nectar, and this could lead to rapid positive reinforcement of 'correct' pollinator behaviour. This would increase floral constancy, and hence also increase pollination efficiency. Furthermore, it is interesting to note that most of the plants with coloured nectar have inflorescences consisting of several to many flowers (e.g. *Hoya* spp., *Aloe* spp., *Schwartzia brasiliensis*, *Leucosceptrum canum*, *Deplanchea tetraphylla*, *Schiedea* spp., *Dombeya* spp., *Grevillea* spp., and *Banksia* spp.). Here, coloured nectar could facilitate a rapid orientation of the pollinators towards rewarding flowers 'in need of' pollen removal or pollen deposition. In turn, this would minimise geitonogamy, and lead to increased outcrossing (de Jong, Waser & Klinkhamer, 1993). A parallel can be drawn to pollinators responding to colour changes in floral parts when flowers are no longer offering rewards; sometimes this colour change is even triggered by a pollinator visiting a flower and harvesting the rewards (Casper & Pine, 1984; Weiss, 1991; Weiss, 1995 and references therein).

In many plants with coloured nectar the signal value of the nectar increases over time. That is, the longer a flower is not visited – and thus the nectar not harvested – the stronger the signal becomes, and in turn the flower might become more attractive to visitors. This can work in at least three different ways that are not mutually exclusive: firstly, in cases where the nectar is strongly coloured and easily seen – the larger the drops, the greater the reward and the stronger the attraction (e.g. *Leucosceptrum canum,* and *Aloe*, *Jaltomata* and *Hoya* spp.). Secondly, the strength of the signal can increase with nectar volume. This is the case in *Schiedea* spp., where the nectar appears black only when a large drop has formed; in small amounts, or spread out thinly, it is a dirty grey and almost translucent (Weller *et al.*, 1995; S. G. Weller personal communication). The same is true for *Trochetia blackburniana*, *T. granulata* and *T. triflora* where the yellow and amber colours of the nectar darken considerably against the corolla with increasing volume. Finally, the nectar colour can change over time. In some of the *Hoya* species, the nectar is initially secreted as a clear or almost clear liquid, which then changes into dark brown or red, perhaps as a result of oxidation (K. F. Yap, personal communication). The same process has been observed in some of the *Aloe* species (Johnson *et al.*, 2006; Nicolson & Nepi, 2005; Reynolds, 1940), in *Jaltomata biflora* (Mione *et al.* 2001), in *Banksia* spp. (Markey & Lamont, 1995), and in some *Deplanchea tetraphylla* flowers (Weber & Vogel, 1986).

However, it is not only the colour of the signal which is important – far more important could be the contrast between signal and background (Burns & Dalen, 2002; Endler, 1992; Schmidt, Schaefer & Winkler, 2004; Hansen *et al.*, 2006). Why, then, do some species with coloured nectar also have strikingly coloured corollas, which could weaken the contrast between nectar and flower? We find this in *Jaltomata weberbaueri*, where the corolla is a bright violet, in contrast to the pale colours in other *Jaltomata* species with coloured nectar. A red corolla with yellow or red nectar is also seen in *Trochetia boutoniana*, *T. uniflora* and *T. blackburniana*. In these species, however, the base of the corolla is often much paler, thus providing a stronger contrast exactly where the nectar drops accumulate (Fig. 2G; Hansen *et al.*, 2006). The same pattern is also seen in *Aloe* section *Anguialoe*. The most extreme case is perhaps *Capsicum pubescens*, where the corolla is almost entirely dark purple, except for five white spots near the centre – which is exactly where the yellow nectar accumulates into visible drops (Fig. 2P). The presence of conspicuous colours in both corolla and nectar may act in a complementary way (Raguso, 2004a). The coloured petals attract visitors from a distance and the coloured nectar then 'fine-tunes' visitor behaviour by guiding them to rewarding flowers. This may be especially important for plant species with large inflorescences with many flowers – which many coloured nectar plants have. Of course, another explanation is that the state is transitory, evolving towards colourful corollas with colourless nectar or *vice versa*.

Overall, these patterns could explain why coloured nectar has evolved repeatedly in habitats with a depauperate pollinator fauna, such as islands and montane areas, where competition for pollinators may be fierce. The relatively long 'shelf-life' of nectar in flowers in such habitats – i.e. potentially it is not 'harvested' that often – may further increase the need to protect this resource against nectar thieves and microbial infestation (see below).

### (5) Other functions of coloured nectar

Floral traits related to pollinator attraction may of course also attract herbivores or nectar robbers (Irwin, Adler & Brody, 2004). Thus, pigments causing colouration of nectar could also be related to defensive or deterring functions, protecting the nectar crop against nectar-thieving ants (Galen, 1983; Stephenson, 1981, 1982), mites (Colwell, 1995), bees (Johnson *et al.*, 2006; Irwin & Brody, 1999) or long-billed nectar-robbing birds (Johnson *et al.*, 2006). Coloured nectar could thus act as a double 'floral filter', keeping inefficient pollinators or nectar robbers at bay, while simultaneously serving as a visual signal for reward to legitimate pollinators – and indeed, this is what Johnson *et al.* (2006) found to be the case for *Aloe vryheidensis* (see Section III.3.*a*). Another unusual nectar-based floral filter is found in *Combretum lanceolatum* (Combretaceae) from Brazil, where the nectar is presented in gelatinous drops that cannot be utilised by flower-visiting insects (Sazima *et al.*, 2001).

Lastly, colour pigments in nectar could serve as agents against microbial infestation (Janzen, 1977; Pichersky & Gershenzon, 2002). Anti-microbial function has been demonstrated for proteins in floral nectars, especially against fungi that could spoil the nectar crop or attack the gynoecium (Carter & Thornburg, 2004; Naqvi *et al.*, 2005). Indeed, Olesen *et al.* (1998) found anti-bacterial properties in the aurone responsible for the red colouration of nectar in *Nesocodon mauritianus*. Also, all *Melianthus* species have very toxic vegetative parts, and extracts from leaves and stems of *Melianthus comosus* have been found to have anti-bacterial activity (Kelmanson, Jäger & van Staden, 2000). While it seems that honey made from *Melianthus* nectar is not toxic to humans (Marloth, 1925), the nectar may still exhibit anti-bacterial properties. Future studies of coloured nectar should investigate potential anti-microbial properties by using bioassays or by screening for compounds with known anti-microbial properties – even though the latter approach could lead to non-detection of hitherto unknown compounds, or of compounds that may be common, but which are not easily screened (Adler, 2000).

The blood-red nectar of *N. mauritianus* provides a cautionary tale: without any ecological evidence, Rosenkranz & Klopman (1999) used a model-based approach to speculate further that the aurone responsible for the red colouration of *N. mauritianus* nectar was a deterrent against endemic Mauritian mammalian nectar robbers. This cannot be the case, as there are no extant or extinct native non-flying mammals in Mauritius (Cheke, 1987a), and the endemic fruitbats – if they ever visited flowers that close to the ground – would most likely be efficient pollen vectors. This example stresses the importance of detailed regional ecological and natural history information when interpreting the potential functions of coloured nectar for a particular plant species. Generally, we need to be cautious in interpreting coloured nectar as adaptive *per se*, whether it is as a signal to attract or deter a certain group of flower-visiting animals, or as an anti-microbial function. We must evaluate each case based on field observations and experimental evidence on a species-by-species basis.

## (6) Non-functional explanations

There are also non-functional explanations for the presence of coloured nectar is some species. For example, in *Melianthus* spp. and *Schwartzia brasiliensis*, the entire plant and the inflorescence, respectively, has dark pigmentation, and the dark colour of the nectar may simply be a non-adaptive or pleiotropic by-product of pigment biosynthesis elsewhere in the plant. A similar argument can be made for *Puya alpestris*, whose petals sport the same blue colour as the nectar, at least in plants grown in gardens, but we need more detailed studies of the plant and its nectar in the wild, where it may be darker and of a more red colouration (C. T. Hornung-Leoni, personal communication). Furthermore, it has recently been investigated how both herbivores and pollinators may select for nectar traits – that is, how traits that are related to both pollinator attractance and herbivore deterrence can be evolutionarily tightly linked (Adler & Bronstein, 2004; Herrera *et al.*, 2002) – and in some species coloured nectar could be an example of such correlated evolution. For *Erythrina*, the unusually high content of amino acids in the nectars of passerine-pollinated *Erythrina* species (Baker & Baker, 1982) could account for the weak colouration of the nectar in the three species, but more investigations across the genus and across individual species' geographical ranges are needed. For *Calliandra calothyrsus* with nocturnal anthesis, it also seems unlikely that the yellow colour of the nectar has any function related to how pollinators perceive the flowers visually.

## (7) Evolution of coloured nectar

The topology of the angiosperm phylogeny (Stevens, 2001 onwards) suggests that coloured nectar has evolved independently at the level of order at least 13 times (Fig. 3), and 15 times at the level of family (Table 1). For the majority of taxa with coloured nectar there are no species-level phylogenies available, and thus we cannot answer questions about single *versus* multiple origins of coloured nectar within these taxa, or speculate on when the trait arose within a lineage. One exception is *Schiedea*, where all four species with coloured nectar form a monophyletic group nested within a well-resolved phylogeny (Soltis *et al.*, 1996; Weller *et al.*, 1995; Wagner *et al.*, 2005). In this case it is most parsimonious to assume that coloured nectar arose once within the clade, most likely in a species from Kaua'i, the older of the two islands where coloured nectar occurs. For *Nesocodon mauritianus*, recent molecular phylogenetic work shows it to be nested within the genus *Heterochaenia* with three species found on the neighbouring island of La Réunion (J. M. Olesen & B. K. Ehlers, unpublished data). The flowers of *H. ensifolia* and *H. rivalsii* have clear nectar, but nectar colour is unknown in *H. borbonica*. Here, we can hypothesise a relatively recent origin of the evolution of coloured nectar, as La Réunion is approximately two million years old (McDougall, 1971). As Mauritius is about eight million years old (McDougall & Chamalaun, 1969), it is thus most likely that *N. mauritianus* is a recent addition to the Mauritian flora, and that coloured nectar evolved here after colonisation from La Réunion during a relatively short time.

Although no well-resolved species-level phylogenies exist for the other taxa with coloured nectar, it is still possible to make inferences about evolutionary events in some of the lineages. In *Hoya*, coloured nectar is found in all five species in the section *Amblyostemma* (Kloppenburg, 1994). This suggests that coloured nectar arose only once in *Hoya*. However, further phylogenetic studies are needed to confirm the monophyly of this section (Wanntorp *et al.*, 2006, Wanntorp *et al.*, in press). Similarly, the *Banksia* species with coloured nectar are all found in one group, *Sphaerocarpa*, in the series *Abietinae* (George, 1999). However, as our knowledge about nectar in this series is incomplete (Markey & Lamont, 1995), and as there is no species-level phylogeny, we cannot deduce anything about single or multiple origins of coloured nectar. In *Jaltomata*, Mione *et al.* (1994) constructed a phylogeny of parts of the

genus, but only two species producing coloured nectar were included. These were members of an unresolved lineage, of which all other species produced clear nectar. Thus, no statement about single *versus* multiple origins of coloured nectar can be inferred here either. Mione & Anderson (1996) speculated that if coloured nectar only evolved once in *Jaltomata*, it could be seen as a key character. Together with long-distance bird dispersal of the brightly coloured berries, it could have fuelled an adaptive radiation of *Jaltomata* species with coloured nectar into various habitats and altitudes.

Similarly, the evolution of black nectar in four *Schiedea* species could be seen as a novel way of attracting birds to provide outcrossing services. The four species with coloured nectar are all autogamous (self-pollinating) and in one case even cleistogamous (the flowers never open) (Weller *et al.*, 1995). However, many other *Schiedea* species show very high levels of inbreeding depression (Culley *et al.*, 1999; Norman *et al.*, 1995; Rankin, Weller & Sakai, 2002; Sakai, Karoly & Weller, 1989; Sakai *et al.*, 1997) and inbreeding depression was recently demonstrated for *S. viscosa* (Weller *et al.*, 2005).

Some of the other taxa with coloured nectar are members of small genera (*Capsicum*, *Leucosceptrum*, *Deplanchea*, *Schwartzia*, *Fernandoa*, and *Lumnitzera*), where almost nothing is known about nectar colour in their respective congeners. Thus, in these cases it is also impossible to state anything about evolutionary events.

In summary, it is either known or suspected that coloured nectar has arisen only once in most lineages. It is not possible, however, to generalise on whether nectar colour is an evolutionarily labile or conservative floral trait. Contrary to the flexibility of floral morphology displayed within most angiosperm lineages, another nectar trait, sugar composition, has been considered a conservative trait (Baker & Baker, 1983; Baker *et al.*, 1998; Nicolson & van Wyk, 1998; van Wyk & Smith, 1996). Thus, we could assume nectar colour to be a conservative trait as well. However, a recent analysis of nectar sugar composition in putative ornithophilous species from the Canary Islands and their closest entomophilous relatives has demonstrated a remarkable evolutionary flexibility in sugar ratios (sucrose:hexose), possibly as a response to selective pressures from generalised avian pollinators (Dupont *et al.*, 2004). Bruneau (1997) reported similar flexibility in sugar ratios and nectar amino acid concentrations in *Erythrina* (Fabaceae), both of which had changed repeatedly to reflect hummingbird- or passerine pollination, irrespectively of phylogenetic relationships. In general, it is thus possible to view nectar colour also as an evolutionarily labile trait, and to interpret shifts in nectar colour as being a result of selective pressures exerted by pollinators. However, phylogenetic inertia rather than continued selective pressures from pollinators could account for the persistence of coloured nectar once it has evolved in a lineage.

Obviously, for a pollinator to exert selective pressure on the colour of nectar, there must be variation in this floral trait in the first place. The vast majority of floral nectars are as clear as water. Therefore, one important question begs answering: how did the original variation in nectar colour in the species with coloured nectar arise? As discussed above, nectar may be coloured for several different reasons – or for no reason at all. Hence, any initial colouration of nectar may have been related to non-signalling functions or be purely pleiotropic in origin. At the moment, we know too little about the ecology and evolution of most taxa with coloured nectar. Only with several species- and lineage-specific studies can this question be addressed, and potential general trends investigated.

## V. CONCLUSIONS AND FUTURE DIRECTIONS

(1) We have shown that coloured nectar is a widespread phenomenon, which is possibly correlated with one or more of several factors: vertebrate pollinators, insularity, and altitude. We believe that the occurrence of coloured nectar is even more widespread than this review suggests. However, coloured nectar is probably often overlooked by taxonomists describing new species, especially if the description is only based on herbarium material. We would thus like to call on workers in plant taxonomy, plant ecology and related fields to pay attention to coloured nectar in their study species and study areas. As shown, coloured nectar may be especially prevalent in vertebrate-pollinated, insular and/or montane taxa. Future reports of additional taxa with coloured nectar will enable us to investigate environmental and ecological correlates with more accuracy, as well as facilitate the testing of current hypotheses and development of new hypotheses on the function and evolution of this spectacular floral trait. With comparative methods, the evolution and maintenance of coloured nectar in lineages could be investigated – but species-level phylogenies are currently only available for a small number of lineages containing species with coloured nectar. Furthermore, future investigations of species with coloured nectar should aim to cover as much of a species' geographical range as possible; some species show variation in expression of nectar colour within a species, and only a broad sampling will elucidate the potential importance of local climatic and edaphic factors, and other nectar properties, such as pH.

(2) Most importantly, there is a great need for experimental studies which address one or more of the hypotheses for the presence of colour pigments in nectar. In relation to the signalling hypothesis, we need studies of plants with coloured nectar and their interactions with flower visitors and pollinators, investigating whether coloured nectar can act as a visual cue and potentially as an honest signal. Experimental approaches could include manipulating nectar colour and amount in real or artificial flowers, and investigating the response of free-ranging or captive flower visitors (*cf.* Hansen *et al.*, 2006; Johnson *et al.*, 2006). Furthermore, future studies should include a biochemical analysis of the nectar to determine the identity of the pigments responsible for the colouration. There is also a need for experimental investigations of other functions of the colour pigments, especially as a defence against microbial infestation, and deterring of flower visitors that are inefficient pollinators or nectar robbers. Ultimately, to demonstrate adaptivity and fitness advantages of coloured nectar in relation to any ecological function, we need experiments in

the field that assess the effect of coloured nectar on reproductive success – i.e. fruit- or seed set.

(3) Already, there is some experimental evidence that coloured nectar can have ecological functions: firstly, in the case of *Aloe* section *Anguialoe*, experiments show that the phenolics which impart the dark colouration of the nectar are distasteful to certain flower visitors that are morphologically unsuitable as pollinators, while at the same time visually attracting birds that are effective as pollinators (Johnson *et al.*, 2006). Secondly, experiments with lizard pollinators in Mauritius show that they strongly prefer coloured nectar to clear nectar in artificial flowers (Hansen *et al.*, 2006).

(4) A caveat in any study of coloured nectar as a visual signal is the 'perception space' (Chittka & Brockmann, 2005) of the flower visitor species that may react to the presence of coloured nectar. Flower visitors are likely to perceive flower- and nectar colour differently to the way humans do, and interpretations of coloured nectar as a visual signal for a certain flower visitor should take the specific visual capabilities of that species into account. A good example of this is our inability to perceive colours in the UV range, whereas many insects, birds and lizards are able to perceive these colours. Hence, future research should also assess nectar colours in the UV range (Thorp *et al.*, 1975). Moreover, as already mentioned, the contrast between colours rather than a preference for specific colours may be important (Hansen *et al.*, 2006; Schmidt *et al.*, 2004). Measurements of nectar and flower colours with a spectrometer (e.g. Ollerton *et al.*, 2003) could be used to quantify colours in a 'perception space'-neutral manner, and comparisons of Euclidian distances (e.g. Schmidt *et al.*, 2004) between flower and nectar colours thus obtained could be made to investigate the importance of colour identity *versus* contrast.

(5) In a review of sexual selection, Andersson & Iwasa (1996) pointed out that sexual selection and signalling studies so far had been a major research area in zoology only, but that future studies could benefit from applying sexual selection and signalling theory in botany as well. Similarly, honest signalling in animals has been an active research area for a long time (see Maynard-Smith & Harper, 2001 and references therein), but it has to our knowledge only been suggested three times previously in plants. Firstly, in relation to nectar scent (Raguso, 2004b) in parallel to our argument in this review. Secondly, in the positive correlation between floral bract size and resin award size in *Dalechampia ipomoeifolia* (Euphorbiaceae) blossoms, and how pollinating bees select flowers based on bract size rather than reward size (Armbruster, Antonsen & Pelabon, 2005). Interestingly, in relation to coloured *versus* clear nectar, Armbruster *et al.* (2005) speculate that while the resin in *D. ipomoeifolia* is clear, it is brightly coloured in many other *Dalechampia* species, and that coloured resin in those species could act as an honest signal without the need for additional honesty via bract size. Thirdly, honest signalling in plants has been proposed in the recent 'autumn colouration' hypothesis of Hamilton & Brown (2001). This hypothesis states that the vibrant autumn leaf colours of deciduous trees are honest signals to deter phytophagous insects, and it has already created substantial debate and led to experimental approaches and calls for more research on colour patterns in plants in general (Atkinson, 2001; Holopainen & Peltonen, 2002; Wilkinson *et al.*, 2002; Hagen, Folstad & Jakobsen, 2003; Schaefer & Wilkinson, 2004; Archetti & Leather, 2005; Schaefer & Rolshausen, 2006). Furthermore, compared to signalling between animals, signals from plants to animals lend themselves well to study, due to a relative constancy in time and space, and a lower complexity and simpler dynamics (Schaefer, Schaefer & Levey, 2004). We propose that plants with coloured nectar and their flower visitors are an excellent study system to investigate honest signalling in plants. Comparative studies in a phylogenetic framework of species with and without coloured nectar would be particularly useful, addressing both ecological and evolutionary aspects of coloured nectar.

(6) In summary, future research on coloured nectar has the potential not only to increase our basic knowledge about this fascinating floral trait, but also to contribute significantly to the diverse fields of signalling theory, nectar biochemistry, pollination biology and the evolutionary ecology of mutualisms.

## VI. ACKNOWLEDGEMENTS

We would like to thank the following people in particular for invaluable help during the long gestation time of this work; for providing information, photos, unpublished data, and/or obscure references: W. Barthlott, A. Boatman, C. Burton, J. R. Chamberlain, J. Christiansen, H. Davis, W.H. Esbaugh, A. Flower, F. Forest, P. Forster, L. Gigord, H. F. Glen, M. Heads, P. B. Heenan, J. Henning, C. T. Hornung-Leoni, K. Jensen, P. Jones, C. N. Kaiser, C. Keena, B. Kemble, R. D. Kloppenburg, T. Krömer, B. B. Lamont, S. Leiva G., D. J. Liddle, H. P. Linder, B. Loison, J. C. Machuca, R. O. Makinson, S. W. Nicolson, T. H. Nielsen, M. V. Norup, T. Nyhuus, J. Ollerton, R. G. Olmstead, R. Pettersson, D. Poole, G. M. Pradhan, C. Puff, R. A. Raguso, D. L. Roberts, M. Rocca, I. & M. Sazima, G. Sankowsky, J. Shykoff, H. Skovgaard, R. W. Thorp, P. B. Tomlinson, D. R. Towns, R. G. Turner, A. Valido, L. van Kradenburg, P. Walin, L. Wanntorp, A. Weber, S. G. Weller, B.-E. van Wyk, P. Wyse Jackson, L. Yacher and K. F. Yap. The manuscript was greatly improved by critical comments and helpful suggestions on earlier drafts by Y. L. Dupont, T. C. Good, S. W. Nicolson, R. A. Raguso, H. M. Schaefer and two anonymous reviewers. Financial support was received from the Swiss National Science Foundation (grant no. 631-065950 to C. B. M.) and from the Carlsberg Foundation and the Danish Natural Research Council (to J. M. O.).

## VII. REFERENCES

Adler, L. S. (2000). The ecological significance of toxic nectar. *Oikos* **91**, 409–420.

Adler, L. S. & Bronstein, J. H. (2004). Attracting antagonists: Does floral nectar increase herbivory? *Ecology* **85**, 1519–1526.

Anderson, S. H. (2003). The relative importance of birds and insects as pollinators of the New Zealand flora. *New Zealand Journal of Ecology* **27**, 83–94.

Andersson, M. & Iwasa, Y. (1996). Sexual selection. *Trends in Ecology and Evolution* **11**, 53–58.

Archer, R. H. & Condy, G. (1997). *Melianthus pectinatus* subsp. *gariepinus*. *Flowering Plants of Africa* **55**, 82–85.

Archetti, M. & Leather, S. R. (2005). A test of the coevolution theory of autumn colours: colour preference of *Rhopalosiphum padi* on *Prunus padus*. *Oikos* **110**, 339–343.

Armbruster, W. S., Antonsen, L. & Pelabon, C. (2005). Phenotypic selection on *Dalechampia* blossoms: Honest signaling affects pollination success. *Ecology* **86**, 3323–3333.

Atkinson, N. (2001). Out on a limb, or a new branch of signalling theory? *Trends in Ecology and Evolution* **16**, 603.

Avé, W. (1984). Deplanchea. *Pacific Plant Areas* **4**, 152–153.

Baker, H. G. & Baker, I. (1973). Amino-acids in nectar and their evolutionary significance. *Nature* **241**, 543–545.

Baker, H. G. & Baker, I. (1983). Floral nectar sugar constituents in relation to pollinator type. In *Handbook of Pollination Biology*, pp. 117–141.

Baker, H. G. & Baker, I. (1986). The occurrence and significance of amino-acids in floral nectar. *Plant Systematics and Evolution* **151**, 175–186.

Baker, H. G. & Baker, I. (1990). The predictive value of nectar chemistry to the recognition of pollinator types. *Israel Journal of Botany* **39**, 157–166.

Baker, H. G., Baker, I. & Hodges, S. A. (1998). Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* **30**, 559–586.

Baker, I. & Baker, H. G. (1982). Some chemical constituents of floral nectars of *Erythrina* in relation to pollinators and systematics. *Allertonia* **3**, 25–37.

Barrett, S. C. H. (1996). The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society London B* **351**, 725–733.

Becerra, J. X. & Lloyd, D. G. (1992). Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): A 2nd action of self-incompatibility at the whole flower level? *Evolution* **46**, 458–469.

Bitter, G. (1921). Solanaceae - Potato family. *Repertorium Specierum Novarum Regni Vegetabilis* **17**, 338–346.

Bitter, G. (1924). Eine Synonymierung. *Hebecladus umbellatus*. *Feddes Repertorium* **20**, 375–376.

Brown, E. D. & Hopkins, M. J. G. (1995). A test of pollinator specificity and morphological convergence between nectarivorous birds and rainforest tree flowers in New Guinea. *Oecologia* **103**, 89–100.

Bruneau, A. (1996). Phylogenetic and biogeographical patterns in *Erythrina* (Leguminosae: Phaseoleae) as inferred from morphological and chloroplast DNA characters. *Systematic Botany* **21**, 587–605.

Bruneau, A. (1997). Evolution and homology of bird pollination syndromes in *Erythrina* (Leguminosae). *American Journal of Botany* **84**, 54–71.

Buchmann, S. L. (1987). The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics* **18**, 343–369.

Buchmann, S. L. & Buchmann, M. D. (1981). Anthecology of *Mouriri myrtilloides* (Melastomataceae, Memecyleae), an oil flower in Panama. *Biotropica* **13**, 7–24.

Burns, K. C. & Dalen, J. L. (2002). Foliage color contrasts and adaptive fruit color variation in a bird-dispersed plant community. *Oikos* **96**, 463–469.

Caihuan, H., Siyi, O., Liang, F., Yong, W., Ning, Z., Zhijie, Q. & Guojun, H. (2004). Study on extraction and stability of pigment from flowers of *Leucosceptrum canum* (Article in Chinese). *Zhong Yao Cai* **27**, 243–246.

Carter, C. & Thornburg, R. W. (2004). Is the nectar redox cycle a floral defense against microbial attack? *Trends in Plant Science* **9**, 320–324.

Carthew, S. M. & Goldingay, R. L. (1997). Non-flying mammals as pollinators. *Trends in Ecology & Evolution* **12**, 104–108.

Casper, B. B. & Pine, T. R. L. (1984). Changes in corolla color and other floral characteristics in *Cryptantha humilis* (Boraginaceae): Cues to discourage pollinators? *Evolution* **38**, 128–141.

Chamberlain, J. R. (1998). Isyzyme variation in *Calliandra calothyrsus* (Leguminosae): Its implications for species delimitation and conservation. *American Journal of Botany* **85**, 37–47.

Chamberlain, J. R. (2000). *Improving seed production in* Calliandra calothyrsus. *A field manual for researchers and extension workers*. Oxford Forestry Institute, Oxford UK.

Cheke, A. S. (1987a). An ecological history of the Mascarene Islands, with particular reference to extinctions and introductions of land vertebrates. In *Studies of Mascarene Island birds* (ed. A. W. Diamond), pp. 5–89. Cambridge University Press, Cambridge.

Cheke, A. S. (1987b). The ecology of the smaller land birds of Mauritius. In *Studies of Mascarene Island birds* (ed. A. W. Diamond), pp. 151–207. Cambridge University Press, Cambridge.

Cheke, R. A. & Mann, C. F. (2001). *Sunbirds: a Guide to the Sunbirds, Flowerpeckers, Spiderhunters and Sugarbirds of the World*. Christopher Helm, London.

Chittka, L. & Brockmann, A. (2005). Perception space – the final frontier. *PLoS Biology* **3**, e137.

Colwell, R. K. (1995). Effects of nectar consumption by the hummingbird flower mite *Proctolaelaps kirmsei* on nectar availability in *Hamelia patens*. *Biotropica* **27**, 206–217.

Court, D. (1981). *Succulent flora of Southern Africa*. A.A. Balkema, Rotterdam.

Cowan, A. M. & Cowan, J. M. (1929). *The trees of Northern Bengal*. Bengal Secretariat Book Depot, Calcutta.

Craig, J. L. & Stewart, A. M. (1988). Reproductive biology of *Phormium tenax* - a honeyeater-pollinated species. *New Zealand Journal of Botany* **26**, 453–463.

Cruden, R. W. (1972). Pollinators in high-elevation ecosystems - relative effectiveness of birds and bees. *Science* **176**, 1439-&.

Culley, T. M., Weller, S. G., Sakai, A. K. & Rankin, A. E. (1999). Inbreeding depression and selfing rates in a self-compatible, hermaphroditic species, *Schiedea membranacea* (Caryophyllaceae). *American Journal of Botany* **86**, 980–987.

Dale, I. R. & Greenway, P. J. (1961). *Kenya trees and shrubs*. Buchanan Estates, Nairobi.

De Jong, T. J., Waser, N. M. & Klinkhamer, P. G. L. (1993). Geitonogamy - the neglected side of selfing. *Trends in Ecology & Evolution* **8**, 321–325.

De Roon, A. C. & Dressler, S. (1997). New taxa of *Norantea* Aubl. s.l. (Marcgraviaceae) from Central America and adjacent South America. *Bot. Jahrb. Syst.* **119**, 327–335.

Dillon, M. O. (1997). Lomas formations: Peru. In *The Americas, vol. 3. Centres of Plant Diversity, A guide and strategy for their conservation* (ed. S. D. Davis *et al.*), pp. 519–527. The World Wide Fund for Nature and IUCN - The World Conservation Union, Oxford, UK.

Dobson, H. E. M. & Bergström, G. (2000). The ecology and evolution of pollen odors. *Plant Systematics and Evolution* **222**, 63–87.

Dortort, F. (2003). Pineapples. Succulent of the Month, July 2003. University of California Botanical Garden. http://

ucbgdev.berkeley.edu/SOM/SOM-pineapples.shtml (accessed January 2006).

Dressler, S. (2004). Marcgraviaceae. In *The Families and Genera of Vascular Plants*, vol. 6 (ed. K. Kubitzki), pp. 258–265. Springer, Berlin.

Dupont, Y. L., Hansen, D. M., Rasmussen, J. T. & Olesen, J. M. (2004). Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology* **18**, 670–676.

Dyer, A. G. (1931). *Aloe sessiliflora. Flowering Plants of South Africa* **11**, Plate 435.

Dyer, A. G. (1941). *Aloe vryheidensis. Flowering Plants of South Africa* **21**, Plate 805.

Dyer, R. A. (1952). *Melianthus villosus. Flowering Plants of Africa* **29**, Plate 1140.

Ehlers, B. K. & Olesen, J. M. (1997). The fruit-wasp route to toxic nectar in *Epipactis* orchids? *Flora* **192**, 1–7.

Elmqvist, T., Cox, P. A., Rainey, W. E. & Pierson, E. D. (1992). Restricted pollination on oceanic islands - pollination of *Ceiba pentandra* by flying foxes in Samoa. *Biotropica* **24**, 15–23.

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist* **139**, S125–S153.

Endress, P. K. (1994). *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press, Cambridge.

Estolas, W. (2004). Green honey produced in 'marijuana capital'. In *Manila Times*, 18. June.

Feinsinger, P., Wolfe, J. A. & Swarm, L. A. (1982). Island ecology: Reduced hummingbird diversity and the pollination biology of plants, Trinidad and Tobago, West Indies. *Ecology* **63**, 494–506.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology Evolution and Systematics* **35**, 375–403.

Forster, P. I. (1992). Pollination of *Hoya australis* (Asclepiadaceae) by *Ocybadistes walkeri sothis* (Lepidoptera: Hesperidae). *Australian Entomological Magazine* **19**, 39–43.

Forster, P. I., Liddle, D. J. & Liddle, I. M. (1998). Diversity in the genus *Hoya* (Asclepiadaceae - Marsdenieae). *Aloe* **35**, 44–48.

Friedmann, F. (1987). 53. Sterculiacées. In *Flore des Mascareignes*, vol. 51-62 (ed. J. Bosser, T. Cadet, J. Guého and W. Marais), pp. 1–50. The Sugar Industry Research Institute, Réduit, Mauritius.

Friis, I. & Bidgood, S. (1998). *Dombeya kefaensis*, sp. nov. (Sterculiaceae) from SW Ethiopia. *Nordic Journal of Botany* **18**, 215–220.

Galen, C. (1983). The effect of nectar-thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. *Oikos* **41**, 245–249.

Gardener, M. C. & Gillman, M. P. (2002). The taste of nectar - a neglected area of pollination ecology. *Oikos* **98**, 552–557.

George, A. S. (1999). Banksia. In *Flora of Australia*, vol. 17B, pp. 175–250. ABRS/CSIRO, Melbourne.

Gigord, L., Lavigne, C. & Shykoff, J. A. (1998). Partial self-incompatibility and inbreeding depression in a native tree species of La Reunion (Indian Ocean). *Oecologia* **117**, 342–352.

Gigord, L., Picot, F. & Shykoff, J. A. (1999). Effects of habitat fragmentation on *Dombeya acutangula* (Sterculiaceae), a native tree on La Reunion (Indian Ocean). *Biological Conservation* **88**, 43–51.

Gill, F. B. (1971). Ecology and evolution of the sympatric Mascarene White-eyes, *Zosterops borbonica* and *Zosterops olivacea*. *Auk* **88**, 36–60.

Givnish, T. J., Millam, K. C., Evans, T. M., Hall, J. C., Pires, J. C., Berry, P. E. & Sytsma, K. J. (2004). Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American-African disjunctions in rapateaceae and bromeliaceae based on ndhF sequence data. *International Journal of Plant Sciences* **165**, S35–S54.

Glen, H. F. & Hardy, D. S. (1995). *Aloe* section Anguialoe and the problem of *Aloe spicata* LF (Aloaceae). *Haseltonia* **3**, 92–103.

Gottsberger, G., Schrauwen, J. & Linskens, H. F. (1984). Amino acids and sugars in nectar, and their putative evolutionary significance. *Plant Systematics and Evolution* **145**, 55–77.

Gourlay, W. B. (1950). *Puya alpestris* in its native land. *Journal of the Royal Horticultural Society* **75**, 399–402.

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology* **144**, 517–546.

Groenewald, B. H. (1938). Beskrywing van 'n nuwe Aloe uit Noord-Transvaal. *Tydskrif vir Wetenskap en Kuns* August, 178–181.

Guillarmod, A. J., Jubb, R. A. & Skead, C. J. (1979). Field studies of six Southern African species of *Erythrina*. *Annals of the Missouri Botanical Garden* **66**, 521–527.

Hagen, S. B., Folstad, I. & Jakobsen, S. W. (2003). Autumn colouration and herbivore resistance in mountain birch (*Betula pubescens*). *Ecology Letters* **6**, 807–811.

Hamilton, W. D. & Brown, S. P. (2001). Autumn tree colours as a handicap signal. *Proceedings of the Royal Society of London Series B Biological Sciences* **268**, 1489–1493.

Hansen, D. M., Beer, K. & Müller, C. B. (2006). Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. *Biology Letters* **2**, 165–168.

Hansen, D. M., Kiesbüy, H. C., Jones, C. G. & Müller, C. B. (in press). Positive indirect interactions between neighbouring plant species via a lizard pollinator. *American Naturalist*.

Hansen, D. M., Olesen, J. M. & Jones, C. G. (2002). Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography* **29**, 721–734.

Hargreaves, A. L., Johnson, S. D. & Nol, E. (2004). Do floral syndromes predict specialization in plant pollination systems? An experimental test in an ''ornithophilous'' African *Protea*. *Oecologia* **140**, 295–301.

Herrera, C. M. (2002). Seed dispersal by vertebrates. In *Plant-animal interactions* (ed. C. M. Herrera and O. Pellmyr), pp. 185–208. Blackwell, Oxford.

Herrera, C. M., Medrano, M., Rey, P. J., Sanchez-Lafuente, A. M., Garcia, M. B., Guitian, J. & Manzaneda, A. J. (2002). Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences* **99**, 16823–16828.

Hesselius, A. (1785). Dissertatio de Aloe. *Dissertationes Academicae Upsaliae Habitae* **2**, 181–194.

Holopainen, J. K. & Peltonen, P. (2002). Bright autumn colours of deciduous trees attract aphids: nutrient retranslocation hypothesis. *Oikos* **99**, 184–188.

Hornung-Leoni, C. & Sosa, V. (2004). Filogenia morphológica de *Puya* subgénero *Puya* (Bromeliaceae). *Primera Reunión Mexicana de Biología Filogenética*, *Xalapa*, Abstract.

Hornung-Leoni, C. & Sosa, V. (2006). Morphological variation in *Puya* (Bromeliaceae): an allometric study. *Plant Systematics and Evolution* **256**, 35–53.

Humeau, L. & Thompson, J. D. (2001). The allometry of flower size dimorphism in dioecious *Dombeya* species on La Réunion. *Ecology Letters* **4**, 221–228.

Irwin, R. E., Adler, L. S. & Brody, A. K. (2004). The dual role of floral traits: Pollinator attraction and plant defense. *Ecology* **85**, 1503–1511.

Irwin, R. E. & Brody, A. K. (1999). Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **80**, 1703–1712.

Jackson, S. M. (2001). Foraging behaviour and food availability of the mahogany glider *Petaurus gracilis* (Petauridae: Marsupialia). *Journal of Zoology* **253**, 1–13.

Janzen, D. H. (1977). Why fruits rot, seeds mold and meat spoils. *American Naturalist* **111**, 691–713.

Jensen, K. (2005). Generalisation and specialisation in tropical pollination systems. MSc thesis, University of Aarhus.

Johow, F. (1898). Über Ornithophilie in der chilenischen Flora. *Sitzungsberichte der Preussischen Akademie der Wissenschaften zu Berlin* **28**, 332–341.

Johnson, S. D, Hargreaves, A. L. & Brown, M. (2006). Dark bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*.

Kalinganire, A., Harwood, C. E., Slee, M. U. & Simons, A. J. (2001). Pollination and fruit-set of *Grevillea robusta* in western Kenya. *Austral Ecology* **26**, 637–648.

Kelmanson, J. E., Jäger, A. K. & van Staden, J. (2000). Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology* **69**, 241–246.

Kessler, M. & Krömer, T. (2000). Patterns and ecological correlates of pollination modes among bromeliad communities of Andean forests in Bolivia. *Plant Biology* **2**, 659–669.

Kleijn, D. & van Donkelaar, R. (2001). Notes on the taxonomy and ecology of the genus *Hoya* (Asclepiadaceae) in Central Sulawesi. *Blumea* **46**, 457–483.

Kloppenburg, D. (1994). *Hoya* sections. *Fraterna*, 1st & 2nd Quarters, pp. I-XXIV.

Knudsen, J. T. & Tollsten, L. (1995). Floral scent in bat-pollinated plants - a case of convergent evolution. *Botanical Journal of the Linnean Society* **119**, 45–57.

Krukoff, B. A. & Barneby, R. C. (1974). Conspectus of species of the genus *Erythrina*. *Lloydia* **34**, 332–459.

Lamont, B. B. (1980). Blue-green algae in nectar of *Banksia* aff. *sphaerocarpa*. *The Western Australian Naturalist* **14**, 193–194.

Lange, R. S., Scobell, S. A. & Scott, P. E. (2000). Hummingbird-syndrome traits, breeding system, and pollinator effectiveness in two syntopic *Penstemon* species. *International Journal of Plant Sciences* **161**, 253–263.

Li, H. W. & Hedge, I. C. (1994). 73. Leucosceptrum. In *Flora of China. Vol. 17 (Verbenaceae to Solanaceae)* (ed. Z. Wu and P. H. Raven), pp. 342. Science Press & Missouri Botanical Garden, St. Louis.

Llamas, K. A. (2003). *Tropical flowering plants: a guide to identification and cultivation*. Timber Press, Cambridge.

Lunau, K. (2000). The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution* **222**, 89–111.

Mabberley, D. J. (1997). *The plant book*. Cambridge University Press, Cambridge.

Makinson, R. O. (2000). Grevillea. In *Flora of Australia*, vol. 17A. ABRS/CSIRO Publishing, Melbourne.

Mann, H. (1866). Revision of the genus *Schiedea* and of the Hawaiian Rutaceae. *Proceedings of the Boston Society of Natural History* **10**, 309–319.

Markey, A. S. & Lamont, B. B. (1995). The potential for cyanobacterial nitrogen fixation in the floral nectar of certain Banksias, pp. 33. Botany Department, University of Western Australia. School of Environmental Biology, Curtin University of Technology, Perth.

Marloth, R. (1925). *The Flora of South Africa. Families 61-106*. Darter Bros. & Co., Capetown.

Martínez Del Rio, C., Baker, H. G. & Baker, I. (1992). Ecological and evolutionary implications of digestive processes - bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia* **48**, 544–550.

Maynard-Smith, J. & Harper, D. (2001). *Animal signals*. Oxford University Press, Oxford.

Mcdougall, I. (1971). The geochronology and evolution of the young volcanic island of Réunion, Indian Ocean. *Geochimica et Cosmochimica Acta* **35**, 261–288.

Mcdougall, I. & Chamalaun, F. H. (1969). Isotopic dating and geomagnetic polarity studies on volcanic rocks from Mauritius, Indian Ocean. *Geological Society of America Bulletin* **80**, 1419–1442.

Mione, T. & Anderson, G. J. (1996). *Jaltomata*: an introduction, and preliminary observations on the red/orange floral nectar. *Solanaceae Newsletter* **4**, 51–57.

Mione, T., Mugaburu, D. & Connolly, B. (2001). Rediscovery and floral biology of Jaltomata biflora (Solanaceae). *Economic Botany* **55**, 167–168.

Mione, T., Olmstead, R. C., Jansen, R. K. & Anderson, G. J. (1994). Systematic implications of chloroplast DNA variation in *Jaltomata* and selected physaloid genera (Solanaceae). *American Journal of Botany* **81**, 912–918.

Muchhala, N. (2003). Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). *Oecologia* **134**, 373–380.

Naqvi, S. M. S., Harper, A., Carter, C., Ren, G., Guirgis, A., York, W. S. & Thornburg, R. W. (2005). Nectarin IV, a potent endoglucanase inhibitor secreted into the nectar of ornamental tobacco plants. Isolation, cloning and characterization. *Plant Physiology* **139**, 1389–1400.

Nicolson, S. W. (1993). Low nectar concentrations in a dry atmosphere - a study of *Grevillea robusta* (Proteaceae) and *Callistemon viminalis* (Myrtaceae). *South African Journal of Science* **89**, 473–477.

Nicolson, S. W. (1995). Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). *Functional Ecology* **9**, 584–588.

Nicolson, S. W. & Nepi, M. (2005). Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). *International Journal of Plant Sciences* **166**, 227–233.

Nicolson, S. W. & Van Wyk, B.-E. (1998). Nectar sugars in Proteaceae: patterns and processes. *Australian Journal of Botany* **46**, 489–504.

Norman, J. K., Sakai, A. K., Weller, S. G. & Dawson, T. E. (1995). Inbreeding depression in morphological and physiological traits of *Schiedea lydgatei* (Caryophyllaceae) in 2 environments. *Evolution* **49**, 297–306.

Nyhagen, D. F., Kragelund, C., Olesen, J. M. & Jones, C. G. (2001). Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. *Journal of Tropical Ecology* **17**, 755–761.

Olesen, J. M. (2003). Island pollinators. In *Island ecosystems: Conservation and molecular approach* (ed. M. A. A. P. d. Carvalho, G. P. Costa, J. A. Jesus and D. M. M. Rodrigues), pp. 45–86. Centre of Biological and Geological Sciences, Funchal.

Olesen, J. M., Eskildsen, L. I. & Venkatasamy, S. (2002). Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions* **8**, 181–192.

Olesen, J. M., & Jordano, P. (2002). Geographic patterns in plant-pollinator mutualistic networks. *Ecology* **83**, 2416–2424.

Olesen, J. M., Rønsted, N., Tolderlund, U., Cornett, C., Mølgaard, P., Madsen, J., Jones, C. G. & Olsen, C. E. (1998). Mauritian red nectar remains a mystery. *Nature* **393**, 529.

Olesen, J. M. & Valido, A. (2003). Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution* **18**, 177–181.

Ollerton, J. & Liede, S. (1997). Pollination systems in the Asclepiadaceae: a survey and preliminary analysis. *Biological Journal of the Linnean Society* **62**, 593–610.

Ollerton, J., Johnson, S. D., Cranmer, L. & Kellie, S. (2003). The pollination ecology of an assemblage of grassland asclepiads in South Africa. *Annals of Botany* **92**, 807–834.

Ortiz-Crespo, F. I. (1973). Field studies on pollination of plants of the genus *Puya*. *Journal of the Bromeliad Society* **23**, 3–7 & 54–58.

Palmer, B., Macqueen, D. J. & Gutteridge, R. C. (1994). *Calliandra calothyrsus* - a multipurpose tree legume for humid locations. In *Forage tree legumes in tropical agriculture* (ed. R. C. Gutteridge and H. M. Shelton), pp. 65–74. CAB International, Wallingford UK.

Pauw, A. (1998). Pollen transfer on birds' tongues. *Nature* **394**, 731–732.

Percival, M. S. (1961). Types of nectar in angiosperms. *New Phytologist* **60**, 235–281.

Pichersky, E. & Gershenzon, J. (2002). The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology* **5**, 237–243.

Pinheiro, M. C. B., Ormond, W. T., De Lima, H. A. & Correia, M. C. R. (1995). Biology of reproduction of *Norantea brasiliensis* Choisy (Marcgraviaceae). *Revista Brasileira de Biologia* **55(Suppl. 1)**, 79–88.

Pole Evans, I. B. (1925). *Aloe sessiliflora*. *Flowering Plants of South Africa* **5**, Plate 180.

Prenner, G. (2002). Secondary pollen presentation on petals of *Dombeya cacuminum* Hochr. (*Sterculiaceae*). *Stapfia* **80**, 323–326.

Proctor, M., Yeo, P. & Lack, A. (1996). *The natural history of pollination*. Timber Press, Portland.

Raguso, R. A. (2004a). Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology* **7**, 434–440.

Raguso, R. A. (2004b). Why are some floral nectars scented? *Ecology* **85**, 1486–1494.

Rankin, A. E., Weller, S. G. & Sakai, A. K. (2002). Mating system instability in *Schiedea menziesii* (Caryophyllaceae). *Evolution* **56**, 1574–1585.

Raven, P. H. (1974). *Eryhthrina* (Fabaceae): Achievements and opportunities. *Lloydia* **37**, 321–331.

Reynolds, G. W. (1940). Genus *Aloe*: A new section and a new series. *Journal of South African Botany* **6**, 111–116.

Richardson, I. B. K. (1979). *Nesocodon mauritianus*. *Kew Bulletin* **33**, 547–550.

Rocca, M. A., Sazima, M. & Sazima, I. (in press). Woody woodpecker enjoys soft drinks: the blond-crested woodpecker seeks nectar and pollinates canopy plants in south-eastern Brazil. *Biota Neotropica*.

Ronse Decraene, L. P., Linder, H. P., Dlamini, T. & Smets, E. F. (2001). Evolution and development of floral diversity of Melianthaceae, an enigmatic Southern African family. *International Journal of Plant Sciences* **162**, 59–82.

Rosenkranz, H. S. & Klopman, G. (1999). The mysterious Mauritian red nectar: A selective toxicant? *Naturwissenschaften* **86**, 404–405.

Ruiz, H. & Pavón, J. (1799). Florae Peruvianae et Chilensis, vol. 2.

Safford, R. J. (1991). Status and ecology of the Mauritius Fody *Foudia rubra* and Mauritius Olive White-eye *Zosterops chloronothos*: two Mauritian passerines in danger. *Dodo* **27**, 113–139.

Sakai, A. K., Karoly, K. & Weller, S. G. (1989). Inbreeding depression in *Schiedea globosa* and *Schiedea salicaria* (Caryophyllaceae), subdioecious and gynodioecious Hawaiian species. *American Journal of Botany* **76**, 437–444.

Sakai, A. K., Weller, S. G., Chen, M. L., Chou, S. Y. & Tasanont, C. (1997). Evolution of gynodioecy and maintenance of females: The role of inbreeding depression, outcrossing rates, and resource allocation in *Schiedea adamantis* (Caryophyllaceae). *Evolution* **51**, 724–736.

Sazima, I., Buzato, S. & Sazima, M. (1993). The bizarre inflorescence of *Norantea brasiliensis* (Marcgraviaceae): visits of hovering and perching birds. *Botanica Acta* **106**, 507–513.

Sazima, M., Sazima, I. & Buzato, S. (1994). Nectar by day and night: *Siphocampylus sulfereus* (Lobeliaceae) pollinated by hummingbirds and bats. *Plant Systematics and Evolution* **191**, 237–246.

Sazima, I., Sazima, C. & Sazima, M. (2005). Little dragons prefer flowers to maidens: a lizard that laps nectar and pollinates trees. *Biota Neotropica* **5**, BN00805012005, http://www.biotaneotropica.org.br/v5n1/pt/fullpaper?bn00805012005+en.

Sazima, M., Vogel, S., do Prado, A. L., de Oliveira, D. M., Franz, G. & Sazima, I. (2001). The sweet jelly of *Combretum lanceolatum* flowers (Combretaceae): a cornucopia resource for bird pollinators in the Pantanal, western Brazil. *Plant Systematics and Evolution* **227**, 195–208.

Schaefer, H. M. & Rolshausen, G. (2006). Plants on red alert: do insects pay attention? *Bioessays* **28**, 65–71.

Schaefer, H. M., Schaefer, V. & Levey, D. J. (2004). How plant-animal interactions signal new insights in communication. *Trends in Ecology & Evolution* **19**, 577–584.

Schaefer, H. M. & Wilkinson, D. M. (2004). Red leaves, insects and coevolution: a red herring? *Trends in Ecology & Evolution* **19**, 616–618.

Schmidt, V., Schaefer, H. M. & Winkler, H. (2004). Conspicuousness, not colour as foraging cue in plant-animal signalling. *Oikos* **106**, 551–557.

Scogin, R. (1985). Floral anthocyanins in the genus *Puya*. *Biochemical Systematics and Ecology* **13**, 387–389.

Scogin, R. & Freeman, C. E. (1984). Floral pigments and nectar constituents in the genus *Puya* (Bromeliaceae). *ALISO* **10**, 617–619.

Scott-Elliot, G. F. (1890). Ornithophilous flowers in South Africa. *Annals of Botany* **4**, 265–280.

Seyani, J. H. (1991). *The genus Dombeya (Sterculiaceae) in Continental Africa*. National Botanical Garden of Belgium, Meise, Belgium.

Sgorbati, S., Labra, M., Grugni, E., Barcaccia, G., Galasso, G., Boni, U., Mucciarelli, M., Citterio, S., Iramategui, A. B., Gonzalez, L. V. & Scannerini, S. (2004). A survey of genetic diversity and reproductive biology of *Puya raimondii* (Bromeliaceae), the endangered queen of the Andes. *Plant Biology* **6**, 222–230.

Sherff, E. E. (1944). Some additions to our knowledge of the flora of the Hawaiian Islands. *American Journal of Botany* **31**, 151–161.

Skead, C. J. (1967). *The sunbirds of Southern Africa*. Balkema, Cape Town.

Smith, L. B. & Downs, R. J. (1974). Pitcairnoideae - Bromeliaceae. *Flora Neotropica* **14**, 1–658.

Soltis, P. S., Soltis, D. E., Weller, S. G., Sakai, A. K. & Wagner, W. L. (1996). Molecular phylogenetic analysis of the Hawaiian endemics *Schiedea* and *Alsinidendron* (Caryophyllaceae). *Systematic Botany* **21**, 365–379.

Sprengel, C. K. (1793). *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen*. Friedrich Vieweg, Berlin.

Staub, F. (1988). Evolutionary trends in some Mauritian phanerogams in relation to their pollinators. *Proceedings of the Royal Society of Arts and Sciences of Mauritius* **5**, 7–78.

Stephenson, A. G. (1981). Toxic nectar deters nectar thieves of *Catalpa speciosa*. *American Midland Naturalist* **105**, 381–383.

Stephenson, A. G. (1982). Iridoid glycosides in the nectar of *Catalpa speciosa* are unpalatable to nectar thieves. *Journal of Chemical Ecology* **8**, 1025–1034.

Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 6, May 2005. URL: http://www.mobot.org/mobot/research/apweb/.

Temeles, E. J. & Kress, W. J. (2003). Adaptation in a plant-hummingbird association. *Science* **300**, 630–633.

Terry, R. G., Brown, G. K. & Olmstead, R. G. (1997). Examination of subfamilial phylogeny in Bromeliaceae using comparative sequencing of the plastid locus *ndh*F. *American Journal of Botany* **84**, 664–670.

Thorp, R. W., Briggs, D. L., Estes, J. R. & Erickson, E. H. (1975). Nectar fluorescence under ultraviolet irradiation. *Science* **189**, 476–478.

Toledo, V. M. (1974). Observations on the relationship between hummingbirds and *Erythrina* species. *Lloydia* **37**, 482–487.

Tomlinson, P. B. (1986). *The botany of mangroves*. Cambridge University Press, Cambridge.

Towns, D. R. (2002). Interactions between geckos, honeydew scale insects and host plants revealed on islands in northern New Zealand, following eradication of introduced rats and rabbits. In *Turning the tide: the eradication of invasive species* (ed. C. R. Veitch and M. N. Clout), pp. 329–335. Invasive Species Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK.

Vahl, M. (1794). *Symbolae botanicae*, Hauniae (Copenhagen).

van der Pijl, L. (1982). *Principles of dispersal in higher plants*. Springer Verlag, Berlin.

van Wyk, B.-E. & Smith, G. (1996). *Guide to the Aloes of South Africa*. Briza Publications, Pretoria.

Viljoen, A. M., Van Wyk, B.-E. & Van Heerden, F. R. (1998). Distribution and chemotaxonomic significance of flavonoids in *Aloe* (Asphodelaceae). *Plant Systematics and Evolution* **211**, 31–42.

Vogel, S. (1998). Remarkable nectaries: Structure, ecology, organophyletic perspectives III. Nectar ducts. *Flora* **193**, 113–131.

Wagner, W. L., Weller, S. G. & Sakai, A. K. (2005). Monograph of *Schiedea* (Caryophyllaceae - Alsinoideae). *Systematic Botany Monographs* **72**, 1–169.

Walsh, B. M. & Hoot, S. B. (2001). Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two non-coding regions: The chloroplast atpB-rbcL spacer region and nuclear waxy introns. *International Journal of Plant Sciences* **162**, 1409–1418.

Wanntorp, L., Kocyan, A. & Renner, S. S. (2006). Wax plants disentangled: A phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **39**, 722–733.

Wanntorp, L., Kocyan, A., van Donkelaar, R. & Renner, S. S. (in press). Towards a monophyletic *Hoya* (Marsdenieae, Apocynaceae): Inferences from the chloroplast trnL region and the rbcL-atpB spacer. *Systematic Botany*.

Ward, N. M. & Price, R. A. (2002). Phylogenetic relationships of Marcgraviaceae: Insights from three chloroplast genes. *Systematic Botany* **27**, 149–160.

Weber, A. & Vogel, S. (1986). The pollination syndrome of *Deplanchea tetraphylla* (Bignoniaceae). *Plant Systematics and Evolution* **154**, 237–250.

Weiss, M. R. (1991). Floral color changes as cues for pollinators. *Nature* **354**, 227–229.

Weiss, M. R. (1995). Floral color change: A widespread functional convergence. *American Journal of Botany* **82**, 167–185.

Weller, S. G. & Sakai, A. K. (1990). The evolution of dicliny in *Schiedea* (Caryophyllaceae), an endemic Hawaiian genus. *Plant Species Biology* **5**, 83–95.

Weller, S. G., Sakai, A. K. & Wagner, W. L. (1995). A phylogenetic analysis of *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae): Implications for the evolution of breeding systems. *Systematic Botany* **20**, 315–337.

Weller, S. G., Sakai, A. K., Wagner, W. L. & Herbst, D. R. (1990). Evolution of dioecy in *Schiedea* (Caryophyllaceae: Alsinoideae) in the Hawaiian Islands: Biogeographical and ecological factors. *Systematic Botany* **15**, 266–276.

Weller, S. G., Sakai, A. K., Rankin, A. E., Golonka, A., Kutcher, B. & Ashby, K. E. (1998). Dioecy and the evolution of pollination systems in *Schiedea* and *Alsinodendron* (Caryophyllaceae: Alsinoideae) in the Hawaiian Islands. *American Journal of Botany* **85**, 1377–1388.

Weller, S. G., Sakai, A. K., Thai, D. A., Tom, J. & Rankin, A. E. (2005). Inbreeding depression and heterosis in populations of *Schiedea viscosa*, a highly selfing species. *Journal of Evolutionary Biology* **18**, 1434–1444.

Wheelwright, N. T. & Janson, C. H. (1985). Colors of fruit displays of bird-dispersed plants in two tropical forests. *American Naturalist* **126**, 777–799.

Whitaker, A. H. (1987). The roles of lizards in New Zealand plant reproductive strategies. *New Zealand Journal of Botany* **25**, 315–328.

Wilkinson, D. M., Sherratt, T. N., Phillip, D. M., Wratten, S. D., Dixon, A. F. G. & Young, A. J. (2002). The adaptive significance of autumn leaf colours. *Oikos* **99**, 402–407.

Willson, M. F. & Whelan, C. J. (1990). The evolution of fruit color in fleshy-fruited plants. *American Naturalist* **136**, 790–809.

Wilson, P., Castellanos, M. C., Hogue, J. N., Thomson, J. D. & Armbruster, W. S. (2004). A multivariate search for pollination syndromes among penstemons. *Oikos* **104**, 345–361.

Wolf, L. L. & Gill, F. B. (1986). Physiological and ecological adaptations of high montane sunbirds and hummingbirds. In *High Altitude Tropical Biogeography* (ed. F. Vuilleumier and M. Monasterio), pp. 103–119. Oxford University Press, Oxford.

Wooller, R. D. & Wooller, S. J. (2003). The role of non-flying animals in the pollination of *Banksia nutans*. *Australian Journal of Botany* **51**, 503–507.

Wyse Jackson, P. S. (1990). *Nesocodon mauritianus* (Campanulaceae). *Kew Magazine* **7**, 113–117.

Zahavi, A. (1975). Mate selection - A selection for a handicap. *Journal of Theoretical Biology* **53**, 205–214.

Zahavi, A. (1977). Cost of honesty (further remarks on handicap principle). *Journal of Theoretical Biology* **67**, 603–605.

## VIII. APPENDIX 1: TABLE REFERENCES

References for Table 1

| | |
|---|---|
| *Aloe alooides* | Glen & Hardy, 1995; S. D. Johnson, unpublished data; B.-E. van Wyk, personal communication; B. Loison, personal communication. |
| *A. castanea* | Reynolds, 1940; Glen & Hardy, 1995; Nicolson & Nepi, 2005; S. D. Johnson, unpublished data. |
| *A. vryheidensis* | Reynolds, 1940; Dyer, 1941; Glen & Hardy, 1995; van Wyk & Smith, 1996; S. D. Johnson, unpublished data. |
| *A. spicata* | Dyer, 1931; Reynolds, 1940; Glen & Hardy, 1995; S. D. Johnson, unpublished data. |
| *Phormium tenax* | Craig & Stewart 1988; Isacch, 2002; J. Henning, personal communication; J. L. Craig, personal communication; P. B. Heenan, personal communication. |
| *Nesocodon mauritianus* | Olesen *et al.*, 1998; Wyse Jackson, 1990. |
| *Schiedea lychnoides* | Weller & Sakai, 1990; Weller *et al.*, 1995; Wagner *et al.*, 2005; S. G. Weller, personal communication. |
| *S. obovatum* | Weller *et al.*, 1995; Wagner *et al.*, 2005; S. G. Weller, personal communication. |
| *S. trinerve* | Weller *et al.*, 1995; Wagner *et al.*, 2005; S. G. Weller, personal communication. |
| *S. viscosum* | Weller *et al.*, 1995; Wagner *et al.*, 2005; S. G. Weller, personal communication. |
| *Schwartzia brasiliensis* | Sazima *et al.* 1993; Pinheiro *et al.* 1995; M. Rocca & M. Sazima, personal communication. |
| *Calliandra calothyrsus* | Palmer *et al.*, 1994; MacQueen & Hernández, 1997; Chamberlain, 1998, 2000; Jensen, 2005; Estolas, 2004. |
| *Erythrina caffra* | Guillarmod *et al.*, 1979; Cheke & Mann, 2001, Scott-Elliot, 1890. |
| *E. humeana* | Guillarmod *et al.*, 1979; Cheke & Mann, 2001. |
| *E. zeyheri* | Guillarmod *et al.*, 1979. |
| *Hoya diversifolia* | Hoffmann *et al.*, 2002; D. Liddle, personal communication; R.D. Kloppenburg, personal communication. |
| *H. excavata* | D. J. Liddle, personal communication; R.D. Kloppenburg, personal communication. |
| *H. kerrii* | Hoffmann *et al.*, 2002; D. J. Liddle, personal communication; R. Pettersson, personal communication; C. Burton, personal communication. |
| *H. meliflua* | Hoffmann *et al.*, 2002; R.D. Kloppenburg, personal communication; D. J. Liddle, personal communication. |
| *H. obovata* | Hoffmann *et al.*, 2002; D. J. Liddle, personal communication. |
| *Melianthus comosus* | Scott-Elliot, 1890; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. dregeanus* | Tansley & Schelpe, 1984; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. elongatus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. gariepinus* | Archer & Condy, 1997; Tansley & Schelpe, 1984; H. P. Linder, unpublished data., unpublished data; J. Henning, personal communication. |
| *M. insignis* | Dyer, 1959; Tansley & Schelpe, 1984; H. P. Linder, unpublished data. |
| *M. major* | Scott-Elliot, 1890; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. pectinatus* | Tansley & Schelpe, 1984; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. villosus* | Dyer, 1952; H. P. Linder, unpublished data; J. Henning, personal communication |
| *Deplanchea tetraphylla* | Avé, 1984; Weber & Vogel, 1986; Brown & Hopkins, 1995; Jackson, 2001. |
| *Fernandoa magnifica* | Weber & Vogel, 1986; Beentje, 1994; Diniz, 1988; C. Puff personal communication. |
| *Leucosceptrum canum* | Cowan & Cowan,1929; Li & Hedge, 1994; Grierson & Long, 1999; G.M. Pradhan, personal communication. |
| *Dombeya a.* ssp. *acutangula* | Gigord *et al.*, 1998, 1999; L. Gigord, personal communication. |
| *D. a.* ssp. *palmata* | L. Gigord, personal communication. |
| *D. a.* ssp. *rosea* | Friedmann, 1987; D. M. Hansen, personal observations. |
| *D. cacuminum* | Prenner, 2002; Llamas, 2003. |
| *D. elegans* | Friedmann ,1987; L. Gigord, personal communication. |
| *D. kefaensis* | Friis & Bidgood, 1998, 2001. |
| *Trochetia blackburniana* | Friedmann , 1987; Olesen *et al.*, 1998; D.M. Hansen & C.B. Müller, unpublished data. |
| *T. boutoniana* | Friedmann , 1987; Olesen *et al.* 1998; D.M. Hansen & C.B. Müller, unpublished data. |
| *T. granulata* | Friedmann, 1987; D.M. Hansen & C.B. Müller, unpublished data. |
| *T. parviflora* | Friedmann, 1987; D.M. Hansen & C.B. Müller, unpublished data. |
| *T. triflora* | Friedmann, 1987; D.M. Hansen & C.B. Müller, unpublished data. |
| *T. uniflora* | Friedmann, 1987; D.M. Hansen & C.B. Müller, unpublished data. |
| *Lumnitzera littorea* | Tomlinson , 1986; H. Skovgaard, personal communication. |
| *Banksia grossa* | Markey & Lamont ,1995; George, 1999; B. B. Lamont, personal communication. |
| *B. incana* | Markey & Lamont, 1995; George, 1999; B. B. Lamont, personal communication. |
| *B. leptophylla* | Markey & Lamont, 1995; George, 1999; B. B. Lamont, personal communication. |
| *B. nutans* | Markey & Lamont, 1995; George, 1999; Wooller & Wooller, 2003; B. B. Lamont, personal communication. |
| *B. sphaerocarpa* | Lamont, 1980; Markey & Lamont, 1995; George, 1999; B. B. Lamont, personal communication. |
| *B. telmatiaea* | Markey & Lamont, 1995; George, 1999; B. B. Lamont, personal communication. |
| *Grevillea robusta* | Skead, 1967; Nicolson, 1993, 1995; Kalinganire, 2000; Makinson, 2000; R. O. Makinson, personal communication. |
| *Capsicum baccatum* | Eshbaugh, 1970; 1975; W. H. Esbaugh, personal communication. |
| *C. pubescens* | Eshbaugh, 1975; W. H. Esbaugh, personal communication; A. Boatman, personal communication. |

References for Table 1: (*cont.*)

| | |
|---|---|
| *C. eximium* | Eshbaugh, 1975; W. H. Esbaugh, personal communication. |
| *Jaltomata aspera* | Bitter, 1921; T. Mione, unpublished data. |
| *J. biflora* | Mione *et al.*, 2001; T. Mione, unpublished data. |
| *J. herrerae* | Mione & Leiva, 1997; T. Mione, unpublished data. |
| *J. paneroi* | Mione & Leiva, 1997; T. Mione, unpublished data. |
| *J. umbellata* | Mione *et al.*, 1993; T. Mione, unpublished data. |
| *J. ventricosa* | Mione *et al.*, 1993; T. Mione, unpublished data. |
| *J. weberbaueri* | T. Mione, unpublished data. |
| *J.* sp. *"contumacencis"* | T. Mione, unpublished data. |
| *J.* sp. *"642"* | T. Mione, unpublished data. |
| *J. sp. "647"* | T. Mione, unpublished data. |
| *J.* sp. *"669"* | T. Mione, unpublished data. |
| *J.* sp. *"711"* | T. Mione, unpublished data. |

References for Table 2:

| | |
|---|---|
| *Aloe castanea* | Nicolson & Nepi, 2005. |
| *A. vryheidensis* | S. D. Johnson *et al.*, unpublished data. |
| *Phormium tenax* | Craig & Stewart 1988. |
| *Nesocodon mauritianus* | Olesen *et al.*,1998; J. M. Olesen, unpublished data. |
| *Schiedea lychnoides* | Weller *et al.*, 1998. |
| *S. obovata* | Weller *et al.*, 1998. |
| *S. trinerve* | Weller *et al.*, 1998. |
| *S. viscosa* | Weller *et al.*, 1998. |
| *Schwartzia brasiliensis* | Sazima *et al.* 1993; Pinheiro *et al.* 1995. |
| *Calliandra calothyrsus* | Jensen, 2005. |
| *Erythrina humeana* | van Wyk, 1993; S. W. Nicolson, personal communication. |
| *E. caffra* | van Wyk, 1993; S. W. Nicolson, personal communication. |
| *Melianthus comosus* | Baker *et al.*, 1998; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. dregeanus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. elongatus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. gariepinus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. major* | Nicolson, 2002; H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. pectinatus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *M. villosus* | H. P. Linder, unpublished data; J. Henning, personal communication. |
| *Deplanchea tetraphylla* | Weber & Vogel, 1986. |
| *Dombeya a.* ssp. *acutangula* | L. Gigord, personal communication. |
| *D. angulata* ssp. *rosea* | D. M. Hansen, unpublished data. |
| *Trochetia blackburniana* | D. M. Hansen & C.B. Müller, unpublished data. |
| *T. boutoniana* | D. M. Hansen & C.B. Müller, unpublished data. |
| *T. granulata* | D. M. Hansen & C.B. Müller, unpublished data. |
| *T. triflora* | D. M. Hansen & C.B. Müller, unpublished data. |
| *T. uniflora* | D. M. Hansen & C.B. Müller, unpublished data. |
| *Lumnitzera littorea* | H. Skovgaard, personal communication. |
| *Banksia leptophylla* | Nicolson & van Wyk, 1998; Markey & Lamont, 1995. |
| *B. sphaerocarpa* | Nicolson & van Wyk, 1998. |
| *B. telmatiaea* | Nicolson & van Wyk, 1998. |
| *Grevillea robusta* | Baker *et al.*, 1998; Kalinganire *et al.*, 2001. |
| *Jaltomata biflora* | Mione *et al.*, 2001. |
| *J. paneroi* | J. M. Olesen & D. M. Hansen, unpublished data. |

## IX. REFERENCES NOT LISTED IN MAIN MANUSCRIPT:


Macqueen, D. J. & Hernandéz, H. M., (1997). A revision of *Calliandra* series *Racemosae* (Leguminosae: Mimosoideae). *Kew Bulletin* **52**, 1–50.

Beentje, H. J. (1994). *Kenya trees, shrubs & lianas*. National Museums of Kenya, Nairobi, Kenya.

Diniz, M. A. (1988). Bignoniaceae. Flora Zambesiaca Volume 8, Part 3.

Dyer, R. A. (1959). *Melianthus insignis*. *Flowering Plants of Africa* **33**, Plate 1310.

Eshbaugh, W. H. (1970). A biosystematic and evolutionary study of *Capsicum baccatum* (Solanaceae). *Brittonia* **22**, 31–43.

Eshbaugh, W. H. (1975). Genetic and biochemical systematic studies of Chili Peppers (*Capsicum* – Solanaceae). *Bulletin of the Torrey Botanical Club* **102**, 396–403.

Friis, I. & Bidgood, S. (2001). Additional observations on *Dombeya kefaensis* (Sterculiaceae) and the diversity of *Dombeya* in SW. Ethiopia. *Nordic Journal of Botany* **21**, 337–340.

Grierson, A. J. C. & Long, D. G. (1999). *Flora of Bhutan. Lamiaceae.* Royal Botanical Garden Edinburgh & Royal Government of Bhutan.

Hoffmann, C., van Donkelaar, R. & Albers, F. (2002). *Hoya*. In *Illustrated handbook of succulent plants. Asclepiadaceae* (ed. F. Albers and U. Meve), pp. 146–158. Springer Verlag, Berlin.

Isacch, J. P. (2002). Nectarivorous feeding by Shiny Cowbirds: A complex feeding innovation. *Wilson Bulletin* **114**, 412–414.

Mione, T., Anderson, G. J. & Nee, M. (1993). *Jaltomata* I: circumscription, description, and new combinations for five South American species (Solaneae, Solanaceae). *Brittonia* **45**, 138–145.

Mione, T. & Leiva, G. S. (1997). A new Peruvian species of *Jaltomata* (Solanaceae) with blood-red floral nectar. *Rhodora* **99**, 283–286.

Nicolson, S. W. (2002). Pollination by passerine birds: why are the nectars so dilute? *Comparative Biochemistry and Physiology Part B* **131**, 645–652.

Tansley, S. A. & Schelpe, E. A. C. L. E. (1984). Melianthaceae. *Bothalia* **15**, 143–145.

van Wyk, B.-E. 1993. Nectar sugar composition in Southern African Papilionoideae (Fabaceae). *Biochemical Systematics & Ecology* **21**, 271–277.

# CHAPTER 2

## Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators

biology letters

*Biol. Lett.* (2006) **2**, 165–168
doi:10.1098/rsbl.2006.0458
*Published online* 1 March 2006

# Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators

**Dennis M. Hansen*, Karin Beer and Christine B. Müller**

*Institute of Environmental Sciences, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland*
**Author for correspondence (dhansen@uwinst.unizh.ch).*

**Most floral nectars are clear as water, and the enigmatic coloured nectar in three endemic plant species in Mauritius has puzzled scientists studying it. One hypothesis about the possible ecological function of coloured nectar is that it serves as a visual signal for pollinators. Recent studies have shown that at least two of the three Mauritian plant species with coloured nectar are visited and pollinated by endemic *Phelsuma* geckos. We here provide experimental evidence for the visual signal hypothesis by showing that *Phelsuma ornata* geckos prefer coloured over clear nectar in artificial flowers. In flowering plants, coloured nectar could additionally function as an honest signal that allows pollinators to assert the presence and judge the size of a reward prior to flower visitation, and to adjust their behaviour accordingly, leading to increased pollinator efficiency. Our study provides a first step in understanding this rare and intriguing floral trait.**

**Keywords:** pollination biology; floral signal; honest signal; nectar properties

## 1. INTRODUCTION

Flowers provide us with countless examples of visual signals intended to be perceived and acted upon by animals visiting the flowers to obtain rewards and to simultaneously provide pollination services. Consequently, pollination biology has provided some of the most striking examples of species interactions in nature. Olesen and co-workers (1998) studied the enigmatic blood-red nectar of the endemic Mauritian plant *Nesocodon mauritianus* (Campanulaceae), and reported coloured nectar in two additional endemic Mauritian species, *Trochetia boutoniana* (red nectar) and *Trochetia blackburniana* (yellow nectar) (Malvaceae). One of their hypotheses was that coloured nectar could act as an honest signal to pollinators, thereby increasing pollination efficiency. They reported two bird species as flower visitors in the only known population of *N. mauritianus*. One species was introduced and the other was a native opportunistic nectar feeder that only acted as a nectar robber. They concluded that a legitimate native pollinator of *N. mauritianus* had not been identified, lamented the fact that it would be hard to pin down lost pollinators among the many extinct animal species of Mauritius, and proclaimed coloured nectar as one of nature's unsolved mysteries.

Since that study, two additional small populations of *N. mauritianus* have been discovered (J.-C. Sevathian & V. Florens, personal communication). Like the first population they are both found on almost vertical cliffs. Unlike that population, however, they are located within the distribution of the diurnal endemic gecko *Phelsuma ornata* (Vinson 1976), which lives in large numbers on the cliffs around the two small populations of *N. mauritianus*. This gecko is a common generalist flower visitor and pollinator in Mauritius (Nyhagen *et al.* 2001; Olesen *et al.* 2002), and it is very likely that it also visits the large, blue flowers of *N. mauritianus*. The two small populations of *N. mauritianus* are both inaccessible, so we were unable to verify gecko visitation or pollination. However, recent research has revealed that the two other Mauritian plant species with coloured nectar, *T. boutoniana* and *T. blackburniana*, are visited and pollinated by *P. ornata* and *Phelsuma cepediana* geckos, respectively (figure 1*a*; D. M. Hansen, H. C. Kiesbüy, C. G. Jones & C. B. Müller, unpublished data).

Thus, Mauritian plants with coloured nectar are both visited and pollinated by endemic *Phelsuma* geckos, which are generalist flower visitors and pollinators in Mauritius (Nyhagen *et al.* 2001; Olesen *et al.* 2002), and which have excellent colour vision (Tanaguchi *et al.* 1999). In our study, we experimentally investigated whether coloured nectar could act as a visual signal to lizard pollinators by experimentally testing the nectar colour preference of *P. ornata* geckos in Mauritius. Our results provide an explanation to the mystery of the Mauritian coloured nectar by demonstrating that *P. ornata* geckos react strongly to coloured nectar as a visual signal for floral reward.

## 2. MATERIAL AND METHODS

*Phelsuma cepediana* is shy, and difficult to observe close-up in the field, whereas *P. ornata* is less shy and often occurs in large numbers. We therefore used *P. ornata* as our study organism, performing experiments on Ile aux Aigrettes, a 25 ha islet with low coastal forest (3–5 m high). There are no plants with coloured nectar on Ile aux Aigrettes, and apart from *Lomatophyllum tormentorii* of which there are only a handful of individuals, none of the gecko-visited plants on the island (see Olesen *et al.* 2002) produce large standing crops of nectar. The geckos on Ile aux Aigrettes are thus naive in relation to nectar colour. We presented pairs of artificial flowers with clear or coloured sugar-water ('nectar') to free-ranging *P. ornata* geckos in their natural habitat. The artificial flowers were constructed by taping four cardboard-petals (0.6 mm thick, in the colours red, yellow, white, green and blue) onto the lower half of 1.5 ml Eppendorf tubes, resulting in 'flowers' of 2.5–3 cm in diameter. The tubes were painted white on the outside to simulate the central white parts of the corollas of *N. mauritianus*, *T. blackburniana* (figure 1*a*) and *T. boutoniana*, where the nectar drops accumulate. Two flowers of the same petal colour were affixed with clear tape to trunks and low branches 1–2 m above ground, with 2–3 cm between the flowers. Both flowers were filled with *ca* 0.5 ml of a 20% sucrose solution, one of which was clear while the other was coloured red or yellow using food colours. The food colours were scentless to a human nose, even in concentrated form. In the wild, *P. ornata* geckos feed on flowers with mainly hexose sugars with concentrations ranging from 5 to 50%, but in preference experiments they showed a preference for sucrose sugar solutions (K. Beer, D. M. Hansen, J. Nüscheler, C. N. Kaiser & C. B. Müller, unpublished data). Flowers were observed from 3–5 m away.

The electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2006.0458 or via http://www.journals.royalsoc.ac.uk.

*Received* 9 December 2005
*Accepted* 1 February 2006

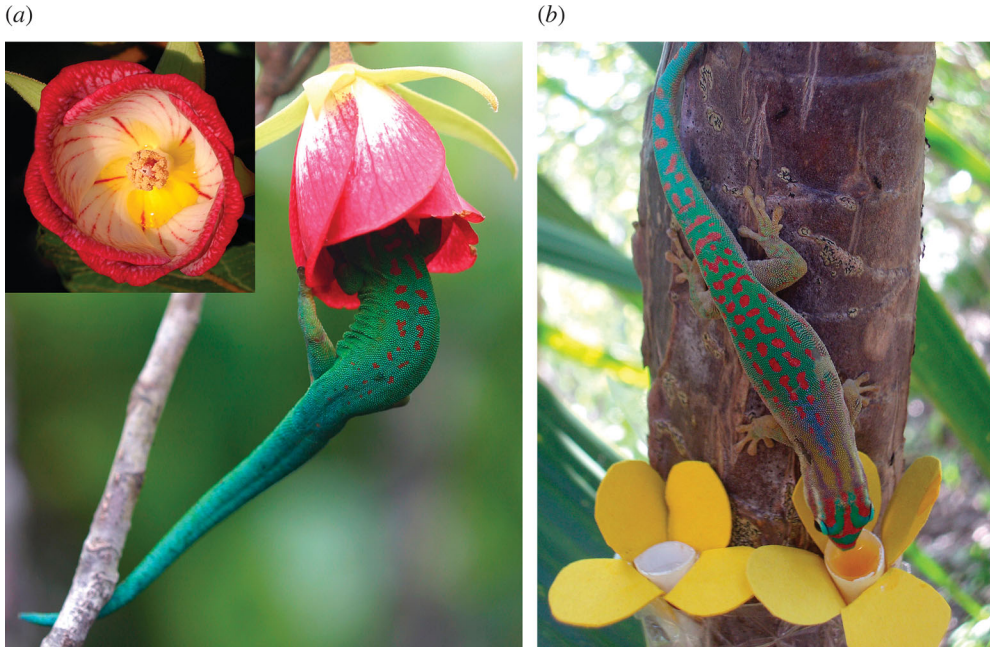



Figure 1. *Phelsuma* geckos and coloured nectar. (*a*) *Phelsuma cepediana* nectar-feeding at *Trochetia blackburniana*. The yellow nectar of this species contrasts well against the white, central part of the otherwise red corolla (insert). (*b*) *Phelsuma ornata* choosing between clear and coloured nectar at experimental flowers.

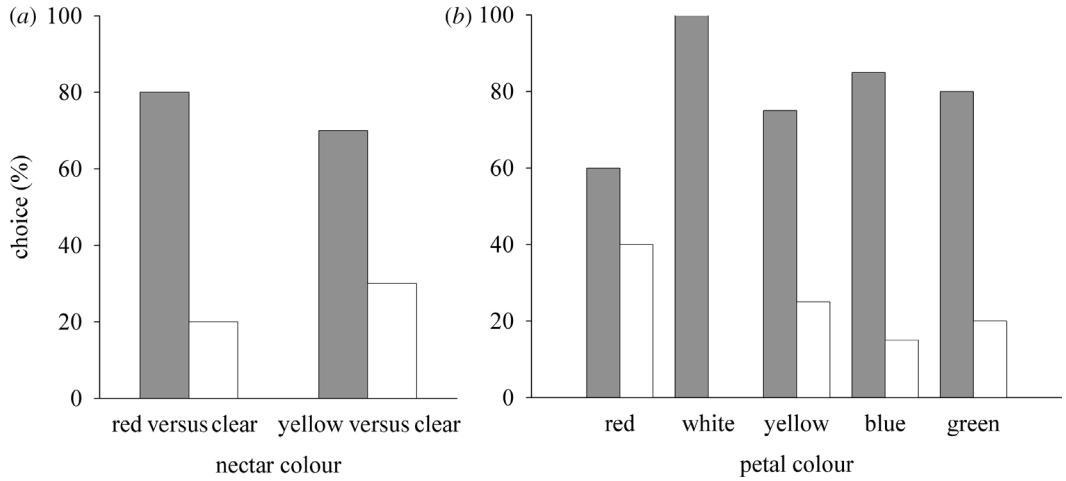



Figure 2. *Phelsuma ornata* nectar colour preference. (*a*) For all five petal colours combined, *P. ornata* prefers red nectar over clear nectar ($\chi^2=36.0$, $p<0.001$, $n=100$), as well as yellow nectar over clear nectar ($\chi^2=8.0$, $p=0.005$, $n=50$). (*b*) *Phelsuma ornata* nectar colour preference for each petal colour separately for red nectar (grey bars) versus clear nectar (white bars). *Phelsuma ornata* significantly prefers red nectar over clear nectar for all petal colours, except red (sign test: red petals, $p=0.503$; white petals, $p<0.001$; yellow petals, $p=0.041$; blue petals, $p=0.003$; green petals, $p=0.012$).

Usually, within 15–30 min, a *P. ornata* gecko would approach the experimental setup, and start licking nectar from one of the flowers. We recorded the flower a gecko approached first as the preferred flower, but only if the approach was from above, so that the nectar was clearly visible and the gecko could choose between flowers. We moved the setup to a new plant at least 2 m away after a gecko's choice had been recorded. We also moved the setup if a gecko approached the flowers from the side or from below, or if there were two or more geckos approaching the flowers simultaneously. The experimental setup was repeated 20 times for red nectar and 10 times each for yellow nectar, in combinations with all five petal colours, for a total of 150 pairwise setups. Although we were not able to discern between individual geckos, it is highly unlikely that the preference of the same gecko was tested twice, i.e. every replicate was likely to be with a different animal.

## 3. RESULTS

On initial approach, a gecko would typically stop at a distance of 30–80 cm from the flowers and remain motionless for anywhere between a couple of seconds and up to several minutes, before the final rapid approach to the chosen flower, where it would start licking the nectar (figure 1*b* and see the electronic supplementary material). The overall picture of preference is very clear: *P. ornata* geckos preferred red and yellow nectar over clear nectar (figure 2*a*; red over clear nectar, $\chi^2=36.0$, $p<0.001$; yellow over clear nectar, $\chi^2=8.0$, $p=0.005$). Analysing the results for each petal colour, we found significant preferences for red over clear nectar for all petal colours, except red (figure 2*b*; sign test: red petals, $p=0.503$; all other petal colours, $p<0.05$; for yellow nectar, the replication of individual petal colours was only 10, rendering statistical analysis meaningless).

## 4. DISCUSSION

Our results provide evidence that coloured nectar in Mauritian plants can function as a visual signal for floral reward to lizard pollinators. Furthermore, because signal and reward are coupled in coloured nectar, it could act as an honest signal by allowing lizards to assert the presence and judge the size of a reward prior to flower visitation, and to adjust their behaviour accordingly, leading to increased pollinator efficiency. For a signal to be honest, there has to be a cost associated with it (*sensu* Zahavi 1977). However, contrary to conventional signalling theory (e.g. Maynard-Smith & Harper 2001), with coloured nectar it is not the presence, nor need it be the production, of the signal that is costly. Rather, it could be the *absence* of the signal that is costly; i.e. when a pollinator has emptied a flower of coloured nectar, this flower will not receive further visits until the signal (and thus the reward) has been replenished. Interestingly, another little-studied nectar trait, scent, was recently proposed to be a potential honest signal, in an argument very similar to ours (Raguso 2004).

Curiously, while the geckos show an absolute preference for red over clear nectar in white flowers, there is no preference for red nectar in red flowers, suggesting that the contrast between nectar and petals is important (Schmidt *et al.* 2004). The fact that the geckos on Ile aux Aigrettes were naive animals that had never previously encountered coloured nectar suggests that the preference for coloured over clear nectar may be innate. Most Mauritian *Phelsuma* species are extremely colourful, with bright red, orange and/or blue colours contrasting strongly against otherwise largely bluish-green scales (figure 1), and it is likely that these colour patterns play an important role in intraspecific behaviour—this is well known from agamid lizards, for example (Madsen & Loman 1987). Hence, the role of *Phelsuma* geckos as important pollinators in Mauritian ecosystems may be facilitated by an innate preference for strong colours or contrasts, combined with their generally inquisitive behaviour.

Of course, coloured nectar in *Trochetia* and *Nesocodon* endemic plants could also be related to other potential pollinators, such as birds. However, the bird most likely to be main pollinators of any of these plants, the specialized nectar-feeding Olive White eye *Zosterops chloronothos*, is critically endangered and thus not easy to study. Furthermore, pigments or substances causing the colouration of the nectar could also be related to other functions, such as defence against nectar robbers or microbial infestation, or simply be a pleitropic effect related to, for example, herbivory defence elsewhere in the plant.

Contrary to Olesen *et al.*'s (1998) assertion, there are more than three plant species in the world with coloured nectar. An upcoming review (D. M. Hansen, J. M. Olesen, T. Mione, S. D. Johnson & C. B. Müller, unpublished data) documents coloured nectar in more than 60 plant species from 14 Angiosperm families worldwide, including several species in the genera *Aloe* (Asphodelaceae) in South Africa (Reynolds 1940), *Schiedea* (Caryophyllaceae) in the Hawaiian archipelago (Weller *et al.* 2005), and *Jaltomata* (Solanaceae) in South America (Mione & Anderson 1996). Many of these species are associated with vertebrate pollinators, and recent experiments confirm that one function of the dark coloured nectar in some South African *Aloe* species is as a visual signal to the most efficient pollinating birds (S. D. Johnson, A. Hargreave & M. Brown unpublished data). Whether coloured nectar functions as a visual signal to pollinators in any of the other plant species remains to be seen. An obvious caveat in any study of coloured nectar as a visual signal is how it relates to the 'perception space' (Chittka & Brockmann 2005) of a given pollinator species. Many pollinators perceive flower- and nectar colours differently to the way humans do. Hence, interpretations of coloured nectar as a visual signal for a certain flower visitor should take the specific visual capabilities of that species into account. Ideally, future studies should employ 'perception-space'-neutral methods, such as spectrometry (e.g. Schmidt *et al.* 2004) to measure specific colour reflectance spectra of flowers and nectars, and relate these to the specific visual capabilities of the pollinator species.

However, to demonstrate adaptiveness and fitness advantages of coloured nectar in relation to any ecological function, experiments in the field that assess the effect of coloured nectar on reproductive success—i.e. fruit- or seed set—are needed. Our study provides a first step in understanding this rare and intriguing floral trait.

We thank J. Nüscheler for help in the field, the Mauritius National Parks & Conservation Service and the Mauritian Wildlife Foundation for support, and C. Kaiser, N. Bunbury, T. Good, C. MacCallum, J. Ollerton and an anonymous reviewer for constructive comments. The work was supported financially by the Swiss National Science Foundation grant no. 631-065950.

Chittka, L. & Brockmann, A. 2005 Perception space—the final frontier. *PLoS Biol.* **3**, 564–568. (doi:10.1371/journal.pbio.0030137)

Madsen, T. & Loman, J. 1987 The role of colour display in the social and spatial organization of male rainbow lizards (*Agama agama*). *Amphib.–Reptil.* **8**, 365–372.

Maynard-Smith, J. & Harper, D. 2001 *Animal signals*. Oxford, UK: Oxford University Press.

Mione, T. & Anderson, G. J. 1996 *Jaltomata*: an introduction, and preliminary observations on the red/orange floral nectar. *Solanaceae Newslett.* **4**, 51–57.

Nyhagen, D. F., Kragelund, C., Olesen, J. M. & Jones, C. G. 2001 Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. *J. Trop. Ecol.* **17**, 755–761. (doi:10.1017/S0266467401001560)

Olesen, J. M., Rønsted, N., Tolderlund, U., Cornett, C., Mølgaard, P., Madsen, J., Jones, C. G. & Olsen, C. E. 1998 Mauritian red nectar remains a mystery. *Nature* **393**, 529. (doi:10.1038/31128)

Olesen, J. M., Eskildsen, L. I. & Venkatasamy, S. 2002 Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Divers. Distrib.* **8**, 181–192. (doi:10.1046/j.1472-4642.2002.00148.x)

Raguso, R. A. 2004 Why are some floral nectars scented? *Ecology* **85**, 1486–1494.

Reynolds, G. W. 1940 Genus *Aloe*: a new section and a new series. *J. S. Afr. Bot.* **6**, 111–116.

Schmidt, V., Schaefer, H. M. & Winkler, H. 2004 Conspicuousness, not colour as foraging cue in plant–animal signalling. *Oikos* **106**, 551–557. (doi:10.1111/j.0030-1299.2004.12769.x)

Taniguchi, Y., Hisatomi, O., Yoshida, M. & Tokunaga, F. 1999 Evolution of visual pigments in geckos. *FEBS Lett.* **445**, 36–40. (doi:10.1016/S0014-5793(99)00089-7)

Vinson, J. M. 1976 The saurian fauna of the Mascarene Islands. II. The distribution of *Phelsuma* species in Mauritius. *Bull. Mauritius Inst.* **8**, 177–195.

Wagner, W. L., Weller, S. G. & Sakai, A. K. 2005 Monograph of *Schiedea* (Caryophyllaceae—Alsinoideae). *Syst. Bot. Monogr.* **72**, 1–169.

Zahavi, A. 1977 Cost of honesty (further remarks on handicap principle). *J. Theor. Biol.* **67**, 603–605.

# CHAPTER 3

## Positive indirect interactions between neighbouring plant species via a lizard pollinator

"...among plants, the nuptials cannot be celebrated without the intervention of a third party to act as a marriage priest, and that the office of this third person is to unite the representatives of different households... ...Now the marriage priests who officiate in the vegetable kingdom are insects in search of honey; the winds, or anything which by accident, or design, may carry the pollen from one flower to another".

- J. T. Rothrock, American Naturalist, vol. 1, 1867.

# Natural History Miscellany

## Positive Indirect Interactions between Neighboring Plant Species via a Lizard Pollinator

**Dennis M. Hansen,[1,*] Heine C. Kiesbüy,[1,†] Carl G. Jones,[2,‡] and Christine B. Müller[1,§]**

1. Institute of Environmental Sciences, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland;
2. Durrell Wildlife Conservation Trust, Les Augres Manor, Trinity, Jersey JE3 5BP, Channel Islands and Mauritian Wildlife Foundation, Grannum Road, Vacoas, Mauritius

*Submitted April 10, 2006; Accepted October 12, 2006; Electronically published January 29, 2007*

*Online enhancements:* appendix figures.

ABSTRACT: In natural communities, species are embedded in networks of direct and indirect interactions. Most studies on indirect interactions have focused on how they affect predator-prey or competitive relationships. However, it is equally likely that indirect interactions play an important structuring role in mutualistic relationships in a natural community. We demonstrate experimentally that on a small spatial scale, dense thickets of endemic *Pandanus* plants have a strong positive trait-mediated indirect effect on the reproduction of the declining endemic Mauritian plant *Trochetia blackburniana*. This effect is mediated by the endemic gecko *Phelsuma cepediana* moving between *Pandanus* thickets, a preferred microhabitat, and nearby *T. blackburniana* plants, where it feeds on nectar and pollinates the plants. Our findings emphasize the importance of considering plant-animal interactions such as pollination at relatively small spatial scales in both basic ecological studies and applied conservation management.

*Keywords:* plant-animal interactions, mutualism, community ecology, facilitation, indirect effects, conservation biology.

* Corresponding author; e-mail: dhansen@uwinst.unizh.ch.

† E-mail: heine@kiesbuy.com.

‡ E-mail: carl.jones@intnet.mu.

§ E-mail: cbm@uwinst.unizh.ch.

Am. Nat. 2007. Vol. 169, pp. 534–542.  0003-0147/2007/16904-41756$15.00. 

> Among plants, the nuptials cannot be celebrated without the intervention of a third party to act as a marriage priest, and that the office of this third person is to unite the representatives of different households. … Now the marriage priests who officiate in the vegetable kingdom are insects in search of honey; the winds, or anything which by accident, or design, may carry the pollen from one flower to another. (J. T. Rothrock, *American Naturalist*, volume 1, 1867)

Most ecological interactions between species are influenced by several co-occurring species because organisms live embedded in interaction networks. Accordingly, theoretical and empirical ecological work has expanded from single- or two-species studies to larger community frameworks of food webs and indirect interactions (e.g., Wootton 1994; Polis and Winemiller 1995; Morin 1999). Most experimental work on indirect interactions between three or more species has focused on either negative effects mediated by natural enemies (Chaneton and Bonsall 2000) or indirect effects occurring along food chains (Schmitz et al. 2000). However, it is equally possible that neighboring species in a community can influence each other positively (Callaway 1997). For example, species can interact positively by providing services or nesting opportunities in return for food or protection against herbivores and predators or by other ways of trading resources (Ollerton 2006).

Pollination biology has provided countless examples of such direct trade in mutualistic interactions (Proctor et al. 1996). However, little is known about the effect of indirect interactions between plants in pollination biology. There are very few studies addressing this specifically; good examples include how neighboring flowering plants affect each other's reproduction negatively (e.g., Waser 1978; Brown and Mitchell 2001; Chittka and Schurkens 2001) or positively (Moeller 2004; Ghazoul 2006) through the specific behavior of shared pollinators. Landscape- or habitat-level differences in pollinator-mediated plant reproductive success have also been investigated in relation to availability of nesting sites, habitat corridors, or habitat

islands for pollinators (Steffan-Dewenter et al. 2001; Townsend and Levey 2005; Artz and Waddington 2006).

Indirect interactions can be classified into those that mediate changes in population densities of the different species involved in trophic consumer-resource interactions (density-mediated or trophic effects; Abrams 1995) and those caused by changes in behavior or other traits between species that are not necessarily trophically dependent (trait-mediated indirect interactions; Abrams 1995). In recent reviews, Werner and Peacor (2003) and van Veen et al. (2006) pointed out the ubiquity and importance of trait-mediated indirect interactions in many different ecosystems, and Schmitz et al. (2004) reviewed the important role of trait-mediated indirect interactions along trophic chains. However, in all reviews, the emphases are again on negative indirect interactions.

In our study, we investigated the role of trait-mediated indirect interactions on a very small spatial scale in a mutualistic pollination system. Specifically, we studied the pollination biology of the endemic plant *Trochetia blackburniana* (Malvaceae) in Mauritius, where preliminary observations had shown that this species was often visited by the endemic blue-tailed day gecko *Phelsuma cepediana* (Gekkonidae). In the study area, dense patches of palmlike *Pandanus* plants (Pandanaceae) are favored microhabitats of this gecko. In our system, then, the trait that is being modified is the behavioral response of *Phelsuma* geckos to the presence of *Pandanus* patches and how the resulting higher density of geckos in and near these patches may confer an indirect positive effect to any gecko-pollinated plants in the vicinity of *Pandanus* patches. That is, our specific hypothesis is that *T. blackburniana* plants growing close to *Pandanus* patches will have a higher gecko visitation rate than plants growing farther away from *Pandanus* patches and that this will result in a correlated higher reproductive success for *T. blackburniana* plants near *Pandanus* patches.

## Material and Methods

### *Study Site and Study Species*

Our study site was Le Pétrin, an upland heath on the island of Mauritius covering some 25 ha in the Black River Gorges National Park, at 670 m above sea level. Le Pétrin is one of the last remnants of a once much more widespread upland heath and marsh that was cleared as late as 1970–1980 and replaced with exotic pine plantations. The upland heath is characterized by poor soils, leading to a low shrubby vegetation of 1–2 m in height, with stunted trees of no more than 5 m in height, interspersed with patches of *Pandanus* plants (Vaughan and Wiehe 1937). Our *Trochetia blackburniana* study plants were all located within a central area of Le Pétrin, covering approximately 15 ha.

*Trochetia blackburniana* belongs, together with five other species, to a genus endemic to Mauritius and La Réunion. Four out of the five Mauritian *Trochetia* species are endangered and occur in only one or a few small relict populations. Only *T. blackburniana* is still relatively widely distributed, albeit in many small, localized populations. Much of its former habitat has been lost, and most of the surviving populations are found in habitats that are rapidly being degraded by invasive species. *Trochetia blackburniana* is a bush, some 1–4 m in height, commonly with a very open, candelabra-like growth form (fig. 1*a*) and large, bell-shaped (3 cm × 2.5 cm) flowers (fig. 1*d*–1*h*). Flowers are protandrous, and the stamens are fused into a tube, with the anthers located at the top. When entering the female phase, the staminal tube falls off, and the style and stigma are revealed underneath (figs. A1, A2 in the online edition of the *American Naturalist*). Because of this dichogamy, levels of autogamy are very low, with only 6.4% fruit set (D. M. Hansen and C. B. Müller, unpublished data), and *T. blackburniana* thus depends almost obligatorily on pollinators to set fruit. The five carpels in each fruit contain a total of 15–30 ovules. *Trochetia blackburniana* has a high level of self-compatibility. Almost all hand-pollinated flowers set fruit, and the resulting seed set is high: selfed flowers have 77% seed set, and outcrossed flowers have 87%–90% seed set (D. M. Hansen and C. B. Müller, unpublished data). Individual flowers last 4–7 days and produce a lot of clear to yellow-orange nectar (fig. A3 in the online edition of the *American Naturalist*). Nectar standing crop at noon is 5–83 $\mu$L (mean $\pm$ SD = 30.9 $\pm$ 17.6 $\mu$L), with 10%–41% sugar content, dominated by hexose sugars (D. M. Hansen and C. B. Müller, unpublished data). An endemic specialist nectarivorous bird, the olive white-eye *Zosterops chloronothos*, has previously been documented as a main flower visitor of *T. blackburniana* (Cheke 1987; Staub 1988; Hansen et al. 2002). However, this bird is critically endangered and still declining (Nichols et al. 2004) and is now locally extinct in most areas where *T. blackburniana* is found, including Le Pétrin. Therefore, we were unable to assess its current importance in the pollination of *T. blackburniana*.

*Pandanus* is the main genus, in terms of distribution and species number, in the Pandanaceae, a large family of trees, shrubs, and climbers that occurs throughout the Old World tropics and subtropics (Cox 1990). For its size, Mauritius harbors a remarkably large number of endemic *Pandanus* species. Before the destructive human impacts, *Pandanus* patches, called *Pandanetums*, were an important structuring feature of the upland plant communities (Vaughan and Wiehe 1937, 1953). However, five out of the 19 endemic species are now extinct, and most of the

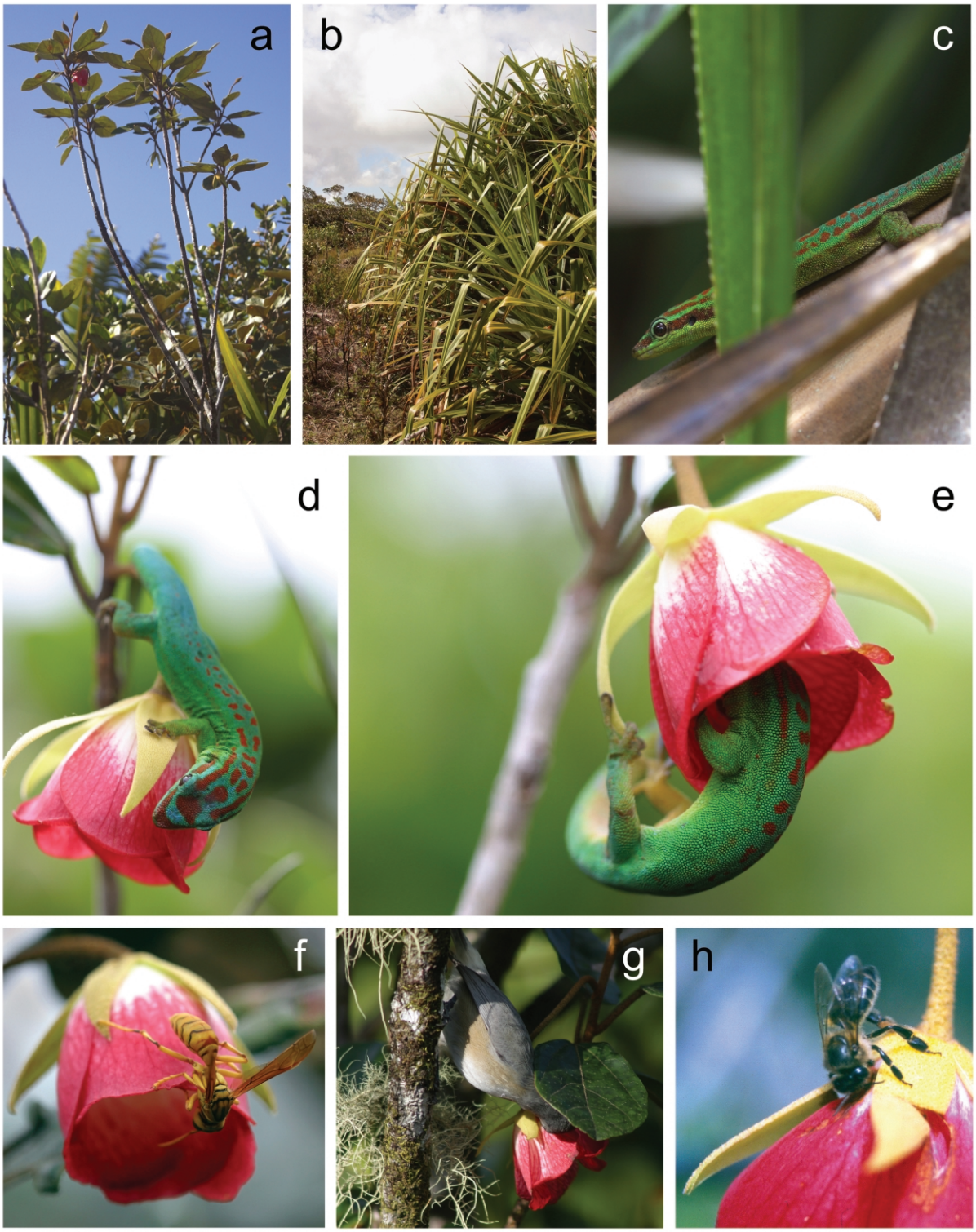



**Figure 1:** Study site and study species. *a*, Typical *Trochetia blackburniana* plant at Le Pétrin. *b*, Edge of a *Pandanus* patch, showing the difference between the relative openness of the heath at Le Pétrin and the dense thicket formed by *Pandanus* plants. *c*, Male *Phelsuma cepediana* gecko moving through the dense maze of spiky *Pandanus* leaves. *d*, Male *P. cepediana* approaching a flower of *T. blackburniana*. *e*, Nectar-feeding male *P. cepediana* in a *T. blackburniana* flower. *f*, Introduced wasp *Polistes hebraeus*. *g*, Endemic Mauritius gray white-eye *Zosterops mauritianus* nectar robbing by piercing a hole at the base of a flower. *h*, Introduced honeybee *Apis mellifera* stealing nectar through a hole pierced by *Z. mauritianus*. Photo *b* by C. N. Kaiser; all other photos by D. M. Hansen.

remaining species are critically endangered through destruction or altering of their habitat (Bosser and Guého 2003). The *Pandanus* patches in our study site were mainly composed of one or several of the following species: *Pandanus barklyi*, *Pandanus palustris*, *Pandanus rigidifolius*, and *Pandanus wiehi*. Because *Pandanus* patches are very variable in size and species composition but otherwise very similar in vegetation structure and leaf morphology, we here define a *Pandanus* patch as a dense stand of one or several *Pandanus* species covering a minimal area of 5 m × 5 m (fig. 1*b*). All *Pandanus* species are dioecious and wind pollinated, and their large (2–10 cm), mostly colorful fruitlets are often dispersed by animals (Cox 1990).

*Phelsuma cepediana* is an endemic diurnal gecko that is common in the remaining upland native plant communities of Mauritius. It is a medium-sized gecko (males = 116–136 mm; females = 77–119 mm total length) with a broad diet of insects, nectar, and fruits (Vinson and Vinson 1969; D. M. Hansen, personal observation). At Le Pétrin and elsewhere in Mauritius, we have observed *P. cepediana* visiting many native and endemic plant species for nectar, including *T. blackburniana* (Hansen et al. 2006; fig. 1*d*, 1*e*). We have no formal density measures of *P. cepediana* in different microhabitats because small arboreal lizards are very difficult to census, but during our work in Le Pétrin, we always saw many more *P. cepediana* in *Pandanus* patches than in any other microhabitat (fig. 1*c*). Even a small patch of *Pandanus* plants forms a dense, impenetrable matrix of spiky, serrated leaves. Hiding in such patches may protect *P. cepediana* from sudden attacks by its main predator, the Mauritian kestrel *Falco punctatus*, a bird feeding almost exclusively on *Phelsuma* geckos (Groombridge et al. 2001), and from other endemic Mauritian birds that prey on *Phelsuma* geckos (Cheke 1987). Furthermore, *Pandanus* patches provide good egg-laying sites, and the dense shade they offer may be important for *Phelsuma* thermoregulation.

### *Pollinator Observations and Fruit Set*

First, it was necessary to establish whether *Phelsuma* visitation rates to *T. blackburniana* plants were indeed affected by proximity to *Pandanus* patches and to investigate whether any other animals visited the flowers. Second, we recorded fruit set for the observed *T. blackburniana* plants. Between March and July 2003, we spent 37 observation periods (mean ± SE = 62.4 ± 2.4 min/period) in Pétrin observing flowering *T. blackburniana* plants. Another flower-visiting endemic Mauritian gecko, *Phelsuma ornata*, in a habitat similar to our study site, was shown to move distances of 0–15 m on average within a 24-h period (Nyhagen et al. 2001). Therefore, we chose to observe *T. blackburniana* plants within the following two distance categories: near (<10 m) or away from (>20 m) *Pandanus* patches. The mean number of flowers observed per period was 12.0 ± 1.36 near *Pandanus* patches and 15.0 ± 1.33 away from *Pandanus* patches. In total, 17 observation periods were spent away from and 20 were near *Pandanus* patches. Many hours of preliminary observations, both close-up and farther away from the plants, revealed no flower visitors smaller than introduced honeybees but did show that birds and geckos in particular were shy when human observers were too close (D. M. Hansen, personal observation). Hence, observations were done from a distance of at least 8 m using 10 × 32-mm binoculars. All observed flower visitors were identified and recorded. To investigate levels of fruit set in relation to animal visitation, we recorded the total number of flowers per plant of all 96 observed plants for the entire flowering season (March–September) and the resulting number of fruits in October 2003, again noting whether the *Trochetia* plants grew near ($n$ = 27 plants) or away from ($n$ = 69 plants) *Pandanus* patches.

### *Gecko Exclusion Experiment*

To test the hypothesis that proximity of *Pandanus* patches leads to higher gecko visitation rates and a subsequently higher fruit set in *T. blackburniana* plants, we performed an exclusion experiment from June to August 2004. We selected 45 experimental plants, to which control plants or parts of plants were assigned. This resulted in a total of 45 pairs that were each assigned a pair ID and used as a random factor in the statistical analysis. Of the 45 experimental plants, 19 grew near (<10 m) and 26 grew away from (>20 m) *Pandanus* patches. To be able to control for possible effects of plant size on reproductive success, we measured the height of all experimental and additional control plants to the nearest centimeter. On the experimental plants of each pair, geckos were excluded from one to three major branches with two to 25 buds (mean ± SE = 7.0 ± 0.59). Exclusion was done by wrapping 30–40 cm of a branch with brown tape and coating this tape with silicon-based car grease. The greased branches and plants were isolated from surrounding vegetation by at least 50 cm because we had observed *P. cepediana* jumping a maximum of 20–30 cm horizontally. Excluded buds on each experimental plant were paired with a similar number of control buds (mean ± SE = 7.2 ± 0.68) on nonexcluded branches of the same plant or adjacent plants (within 5 m) of similar size (total additional control plants $n$ = 19; nine near and 10 away). The 19 experimental plants that grew near *Pandanus* patches occurred in five distinct groups, each of which grew within separate clusters of *Pandanus* patches at least 150 m apart (two to seven experimental *T. blackburniana* plants and between five and

eight *Pandanus* patches per cluster). The 26 experimental plants that grew away from *Pandanus* patches were selected in five separate groups covering an area similar to the five clusters of *Pandanus* patches. These 10 groups were assigned a cluster ID that was used as a random factor in the statistical analysis.

After 5–7 weeks, in August 2004, developing fruits were counted and harvested. Because of previously observed high levels of fruit and seed predation by introduced rats and lepidopteran larvae throughout the *T. blackburniana* population in Pétrin (D. M. Hansen, personal observation), we harvested unripe fruits. It is easy to discern developing fruits because the previously flexible peduncle goes rigid and the fruit orients itself upward within a week of fertilization. In contrast, a flower that is not fertilized will rapidly wilt and fall off shortly after anthesis. The unripe fruits were cut open with a scalpel to score the number of developing seeds and the total number of ovules. Only fruits where we could clearly discern between small, unfertilized ovules and larger, developing seeds were included in the seed set analysis.

### *Statistical Analyses*

Measured variables are all presented as means ± SE. Visitation rates to *T. blackburniana* in relation to proximity of *Pandanus* patches were analyzed by ANOVA after a $(x + 1)^{-2}$ transformation to obtain normal distribution of residuals. Fruit set data from the first season were analyzed with a generalized linear model using a quasi-binomial error structure, with proximity to *Pandanus* as an explanatory variable. For the analysis of fruit set in the second experimental season, because of the overall unbalanced design and many zero values and to account for the spatial structure of clusters, we fitted a generalized linear mixed-effects model (GLMM in GenStat 9.1 with a fixed fitting; Breslow and Clayton 1993) using a binomial error structure. Proximity to *Pandanus* patches and exclusion treatment versus control plants were fixed effects; pair ID nested in cluster ID were random effects. We obtained complete seed set data for only a very small subset of pairs. Hence, the analysis of seed set was done by comparing average seed set at the pair level with an ANOVA, with proximity to *Pandanus* patches and exclusion treatment versus control plants as explanatory variables. Apart from the GLMM, all analyses were done using R, version 2.3.1 (R Development Core Team 2006).

## Results

### *Pollinator Observations and Fruit Set*

At *Trochetia blackburniana* flowers, we observed *Phelsuma cepediana* (fig. 1*d*, 1*e*), the introduced wasp *Polistes hebraeus* (fig. 1*f*), the endemic bird Mauritius gray white-eye *Zosterops mauritianus*, and the introduced honeybee *Apis mellifera*, all of which were foraging for nectar. The latter two almost exclusively foraged for nectar through holes pierced in the corolla and are therefore unlikely to be efficient pollen vectors (fig. 1*g*, 1*h*). When *P. cepediana* entered the flowers, it did so either from above, climbing down the peduncle, or by reaching out to grab and enter the flower while still clinging onto the branch (fig. A1*b*, A1*c*; fig. A4 in the online edition of the *American Naturalist*). In doing so, pollen was deposited either just behind the head or on the gecko's throat and chest. There was a significant difference in visitation rate between visitor species ($F = 34.459$, df $= 1, 140$, $P < .001$) and a significant effect of proximity to *Pandanus* patches ($F = 22.271$, df $= 1, 140$, $P < .001$). Most important, there was a significant interaction between flower visitor species and distance ($F = 16.197$, df $= 3, 140$, $P < .001$), which resulted from the much higher mean visitation rate of *P. cepediana* when *T. blackburniana* grew near *Pandanus* patches compared to that for plants farther away (fig. 2*a*). The resulting fruit set of observed *T. blackburniana* plants in September 2003 was significantly increased when plants grew near *Pandanus* patches ($F = 29.004$, df $= 1, 94$, $P < .001$; fig. 2*b*).

### *Gecko Exclusion Experiment*

Gecko exclusion had significant effects on the fruit set of *T. blackburniana* (fig. 3); there was a highly significant negative effect of gecko exclusion on the fruit set of *T. blackburniana* (df = 1, Wald/df = 42.88, $P < .001$) and a significant difference in fruit set near and away from *Pandanus* patches (df = 1, Wald/df = 9.07, $P = .003$). However, there was no significant interaction between proximity and gecko exclusion (df = 1, Wald/df = 0.32, $P = .574$), meaning that fruit set on excluded branches was decreased both near and away from *Pandanus* patches.

This is probably due to the fact that the exclusion experiment was carried out in the peak flowering time of *T. blackburniana*, which is also a time when *P. cepediana* is very active. Thus, even away from *Pandanus* patches, we get a significant effect on fruit set by excluding geckos. There was no difference in seed set of fruits developing from excluded and control flowers or from fruits near and away from *Pandanus* patches ($F = 0.2806$, df $= 2, 33$, $P = .757$). Overall average seed set was 43.8% ± 0.04%. Compared with the 70%–80% seed set from hand pollination, it seems that *T. blackburniana* is pollen limited—even in control plants that grow near *Pandanus* patches.

There was no significant difference in mean height of experimental and control plants near and away from

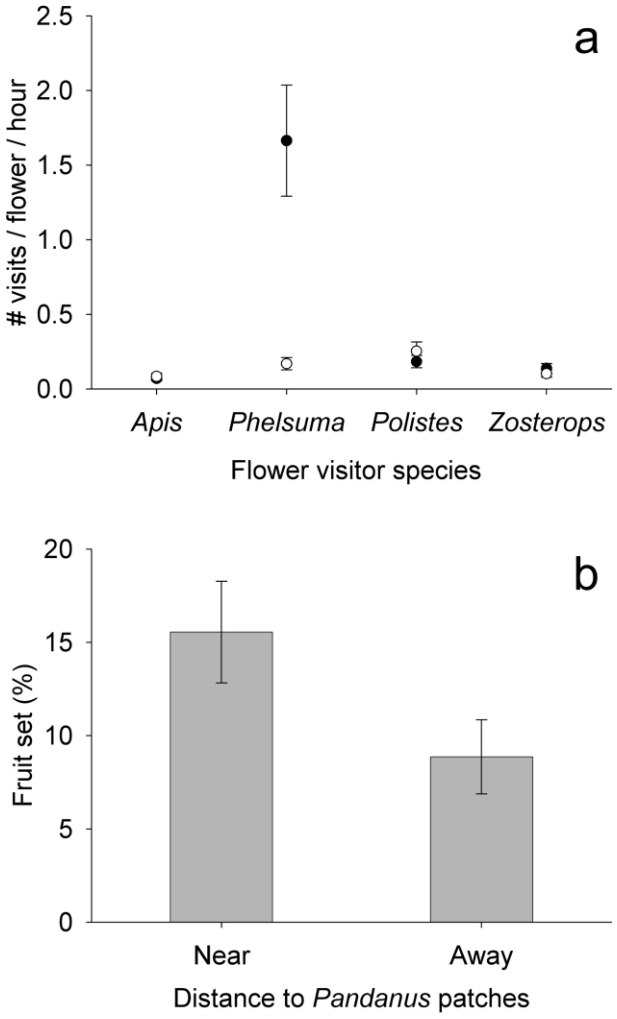



**Figure 2:** *a*, Visitation rates (mean ± 1 SE) of all flower visitors at *Trochetia blackburniana* flowers that grow near (*filled circles*) and away from (*open circles*) *Pandanus* patches. The visitation rate of only the gecko *Phelsuma cepediana* is dramatically increased near *Pandanus* patches. *b*, Subsequent fruit set (mean ± 1 SE) is significantly higher for *T. blackburniana* plants growing near *Pandanus* patches compared to that of plants growing farther away.

*Pandanus* patches (near = 159.0 ± 8.9 cm; away = 155.2 ± 10.7 cm; $t = 0.27$, $P = .788$).

## Discussion

We found a strong positive correlation between proximity of *Trochetia blackburniana* plants to *Pandanus* patches and *Phelsuma* visitation rates, which was mirrored in the resulting fruit set of *T. blackburniana*. The gecko exclusion experiment confirmed this pattern, providing evidence of a positive trait-mediated indirect interaction between two unrelated plant species via a pollinator of only one of them. Thus, plant community structure combined with pollinator behavior on a very small spatial scale are strong determinants for the reproductive success of *T. blackburniana*. It should be noted that the overall lower fruit set illustrated in figure 2, compared to that in figure 3, is due to the former being based on the whole flowering season, from March to September, while the latter is based on 5 weeks in the peak flowering season, when the geckos are most active as flower visitors. Hence, the pattern of high fruit set near *Pandanus* is much clearer when based on flowers that are open during the peak flowering season of *T. blackburniana*.

Of course, there could be other explanations for the observed difference in reproductive success between *T. blackburniana* plants close to and away from *Pandanus* patches. One possibility would be corresponding differences in soil nutrients. However, in another study at Le Pétrin (C. N. Kaiser and C. B. Müller, unpublished data), an analysis of soil samples from six transects of 100 m, spaced 60–75 m apart, revealed no significant differences in nutrient levels between transects. Several of our observed plants ($n \approx 50$) and experimental/control plants ($n = 22$), as well as many of the *Pandanus* patches (together forming two of the five clusters used in our analysis), grew within this area. Furthermore, if there would have been differences in soil nutrients between near and away that affected growth and vigor of *T. blackburniana* plants, we would not have expected to find a uniform plant size distribution across the population at Le Pétrin, nor would we have expected to find such a strong effect

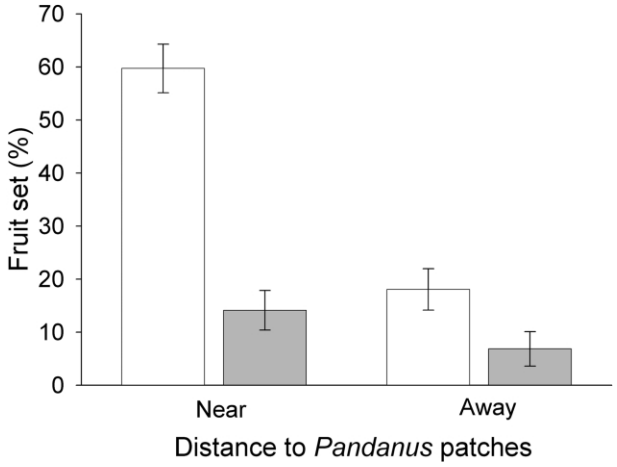



**Figure 3:** Fruit set (proportion of flowers developing into fruits; mean ± 1 SE) of experimental *Trochetia blackburniana* plants is correlated to proximity of *Pandanus* patches. Open bars refer to the fruit set of control flowers and shaded bars to the fruit set of flowers from which geckos were excluded.

on *T. blackburniana* reproductive success by the simple experiment of excluding nonflying pollinators (i.e., geckos) near *Pandanus* patches.

Our study is a good example of how trait-mediated indirect interactions can not only structure predator-prey or competitive interactions but also play a significant role in mutualistic interactions. Other studies have also shown pollinator-mediated positive indirect interactions between plant species on a small spatial scale (Thomson 1978; Dafni 1983; Laverty 1992; Johnson et al. 2003; Moeller 2004; Ghazoul 2006), but in all of these cases, the facilitation results from shared pollinators seeking rewards in two or more plant species that were often closely related. In our system, the positive indirect effect between two plant species is mediated by an animal that seeks floral rewards in—and acts as pollinator of—only one of them. Moreover, our results highlight the significance of the community context when considering conservation management of endangered plant species.

Lizard pollination of *T. blackburniana* is an interesting phenomenon in itself because only a few studies so far have identified lizards as important pollinators of plants. Most of the known examples of lizard pollination occur on islands where a low diversity and a low abundance of invertebrates may force otherwise mostly insectivorous lizards to expand their diet to include fruit and nectar (Olesen and Valido 2003). Insular lizard species also often occur in extremely high densities compared to congeners in mainland habitats (Rodda and Dean-Bradley 2002), which in turn is likely to increase their relative importance in insular food webs. Another Mauritian gecko, *Phelsuma ornata*, has previously been shown to be an efficient pollen vector for several coastal plants (Nyhagen et al. 2001). Furthermore, Olesen et al. (2002) found *P. ornata* to be the most important endemic species in a pollination network on the Mauritian offshore islet of Île aux Aigrettes. An interesting point, which we were sadly unable to address in our study, is the potential role of colored nectar in *Phelsuma-Trochetia* interactions. A recent study by Hansen et al. (2006) demonstrated how the mysterious Mauritian colored nectar (Olesen et al. 1998) acts as a visual floral signal for *Phelsuma* geckos. Because the nectar color of *T. blackburniana* varies from clear to yellow-orange, interplant or seasonal differences in nectar color may play a structuring role in our system as well.

There is evidence that proximity to *Pandanus* patches affects gecko visitation rate and reproductive success in at least two other endemic Mauritian plants, *Labourdonnaisia callophylloides* (Sapotaceae; C. N. Kaiser and C. B. Müller, unpublished data) and the critically endangered *Roussea simplex* (Rousseaceae; D. M. Hansen and C. B. Müller, unpublished data). Hence, our results lead to valuable management insights for ongoing conservation efforts to save the highly endangered flora of Mauritius. Additionally, large numbers of *Phelsuma* geckos in *Pandanus* plants have also been noted in the neighboring island of La Réunion (L. Gigord, personal communication) and in Madagascar (M. Callmander, personal communication). Both these islands are also biodiversity hotspots with high levels of endemism and many endangered plant taxa, so our results may be applicable there as well.

*Pandanus* plants and thickets may be important for species-level and functional biodiversity in many habitats in the paleotropics, where they are being used by a wide variety of animals as nesting places, homes, or hideouts (e.g., birds and small marsupials in Australia [Braithwaite and Lonsdale 1987; Rowley and Russell 1993] and freshwater crabs in Taiwan [Schubart et al. 2003] and Madagascar [Cumberlidge et al. 2002]). Lehtinen (2002) documented a total of 41 species of reptiles and amphibians in Madagascar, including five *Phelsuma* species, that were frequently or obligatorily associated with *Pandanus* plants. For some of the above species, loss of *Pandanus* patches resulted in population declines (Braithwaite and Lonsdale 1987; Schubart et al. 2003), again demonstrating the importance of *Pandanus* patches for conservation management. Together with our results, these studies demonstrate that the habitat heterogeneity caused by dense *Pandanus* thickets can promote complex community interactions for a wide range of species across much of the paleotropics.

To conclude, trait-mediated indirect interactions have received much recent attention in community ecological studies of predator-prey and competitive interactions reviewed by Schmitz et al. (2004) and Werner and Peacor (2003). Here, we demonstrate an equal importance of trait-mediated indirect interactions in structuring a mutualistic pollination interaction. Moreover, given that lizard pollination is widespread on many islands, and given that islands harbor much of the world's endangered flora and herpetofauna, our results are relevant for the conservation of many endangered island plants and lizards. Our findings emphasize the importance of considering plant-animal interactions such as pollination at relatively small spatial scales in both basic ecological studies and applied conservation management.

## Acknowledgments

We thank the National Parks and Conservation Service of Mauritius and the Mauritian Wildlife Foundation for their support; L. Reiter for help in the field; C. Kaiser for use of a photo; R. Callaway, E. Conti, T. Good, A. Hector, C. Kaiser, J. Krauss, R. Mitchell, L. Turnbull, and two reviewers for constructive comments; and S. Härri, A. Hector, and C. Philipson for help with the statistical analyses.

The Swiss National Science Foundation (grant 631-065950 to C.B.M.) financed the study.

## Literature Cited


Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. American Naturalist 146:112–134.

Artz, D. R., and K. D. Waddington. 2006. The effects of neighbouring tree islands on pollinator density and diversity, and on pollination of a wet prairie species, *Asclepias lanceolata* (Apocynaceae). Journal of Ecology 94:597–608.

Bosser, J., and J. Guého. 2003. Pandanacées. Flore des Mascareignes. Vol. 190. Mauritius Sugar Industry Research Institute, Reduit.

Braithwaite, C. J. R., and W. M. Lonsdale. 1987. The rarity of *Sminthopsis virginae* (Marsupialia: Dasyuridae) in relation to natural and unnatural habitats. Conservation Biology 1:341–344.

Breslow, N. E., and D. G. Clayton. 1993. Approximate inference in generalized linear mixed models. Journal of the American Statistical Association 88:9–25.

Brown, B. J., and R. J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia (Berlin) 129:43–49.

Callaway, R. M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. Oecologia (Berlin) 112: 143–149.

Chaneton, E. J., and M. B. Bonsall. 2000. Enemy-mediated apparent competition: empirical patterns and the evidence. Oikos 88:380–394.

Cheke, A. S. 1987. The ecology of the smaller land birds of Mauritius. Pages 151–207 *in* A. W. Diamond, ed. Studies of Mascarene Island birds. Cambridge University Press, Cambridge.

Chittka, L., and S. Schurkens. 2001. Successful invasion of a floral market. Nature 411:653.

Cox, P. A. 1990. Pollination and the evolution of breeding systems in Pandanaceae. Annals of the Missouri Botanical Garden 77:816–840.

Cumberlidge, N., C. B. Boyko, and A. W. Harvey. 2002. A new genus and species of freshwater crab (Crustacea, Decapoda, Potamoidea) from northern Madagascar, and a second new species associated with *Pandanus* leaf axils. Journal of Natural History 36:65–77.

Dafni, A. 1983. Pollination of *Orchis caspia*: a nectarless plant which deceives the pollinators of nectariferous species from other plant families. Journal of Ecology 71:467–474.

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology 94:295–304.

Groombridge, J. J., M. W. Bruford, C. G. Jones, and R. A. Nichols. 2001. Evaluating the severity of the population bottleneck in the Mauritius kestrel *Falco punctatus* from ringing records using MCMC estimation. Journal of Animal Ecology 70:401–409.

Hansen, D. M., J. M. Olesen, and C. G. Jones. 2002. Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? Journal of Biogeography 29:721–734.

Hansen, D. M., K. Beer, and C. B. Müller. 2006. Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. Biology Letters 2:165–168.

Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Agren. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. Ecology 84:2919–2927.

Laverty, T. M. 1992. Plant interactions for pollinator visits: a test of the magnet species effect. Oecologia (Berlin) 89:502–508.

Lehtinen, R. M. 2002. The use of screw pines (*Pandanus* spp.) by amphibians and reptiles in Madagascar. Herpetological Bulletin 82:20–25.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology 85:3289–3301.

Morin, P. 1999. Community ecology. Blackwell Science, Oxford.

Nichols, R., L. Woolaver, and C. Jones. 2004. Continued decline and conservation needs of the endangered Mauritius olive white-eye *Zosterops chloronothos*. Oryx 38:291–296.

Nyhagen, D. F., C. Kragelund, J. M. Olesen, and C. G. Jones. 2001. Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. Journal of Tropical Ecology 17: 755–761.

Olesen, J. M., and A. Valido. 2003. Lizards as pollinators and seed dispersers: an island phenomenon. Trends in Ecology & Evolution 18:177–181.

Olesen, J. M., N. Rønsted, U. Tolderlund, C. Cornett, P. Mølgaard, J. Madsen, C. G. Jones, and C. E. Olsen. 1998. Mauritian red nectar remains a mystery. Nature 393:529.

Olesen, J. M., L. I. Eskildsen, and S. Venkatasamy. 2002. Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. Diversity and Distribution 8:181–192.

Ollerton, J. 2006. "Biological barter": patterns of specialization compared across different mutualisms. Pages 411–435 *in* N. M. Waser and J. Ollerton, eds. Plant pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago.

Polis, G. A., and K. Winemiller. 1995. Food webs: integration of patterns and dynamics. Chapman & Hall, New York.

Proctor, M., P. Yeo, and A. Lack. 1996. The natural history of pollination. Timber, Portland.

R Development Core Team. 2006. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rodda, G. H., and K. Dean-Bradley. 2002. Excess density compensation of island herpetofaunal assemblages. Journal of Biogeography 29:623–632.

Rothrock, J. T. 1867. The fertilization of flowering plants. American Naturalist 1:64–72.

Rowley, I., and E. Russell. 1993. The purple-crowned fairy-wren *Malurus coronatus*. 2. Breeding biology, social-organization, demography and management. Emu 93:235–250.

Schmitz, O. J., P. A. Hambäck, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. American Naturalist 155:141–153.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters 7:153–163.

Schubart, C. D., H. C. Liu, and J. A. Cuesta. 2003. A new genus and new species of tree-climbing crab (Crustacea: Brachyura: Sesarmidae) from Taiwan with notes on its ecology, larval morphology and phylogeny. Raffles Bulletin of Zoology 51:49–59.

Staub, F. 1988. Evolutionary trends in some Mauritian phanerogams in relation to their pollinators. Proceedings of the Royal Society of Arts and Sciences of Mauritius 5:7–78.

Steffan-Dewenter, I., U. Munzenberg, and T. Tscharntke. 2001. Pollination, seed set and seed predation on a landscape scale. Proceedings of the Royal Society B: Biological Sciences 268:1685–1690.

Thomson, J. D. 1978. Effects of stand composition on insect visitation in two-species mixtures of *Hieracium*. American Midland Naturalist 100:431–440.

Townsend, P. A., and D. J. Levey. 2005. An experimental test of whether habitat corridors affect pollen transfer. Ecology 86:466–475.

van Veen, F. J. F., R. J. Morris, and H. C. J. Godfray. 2006. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annual Review of Entomology 51: 187–208.

Vaughan, R. E., and P. O. Wiehe. 1937. Studies on the vegetation of Mauritius. I. A preliminary survey of the plant communities. Journal of Ecology 25:289–343.

———. 1953. The genus *Pandanus* in the Mascarene Islands. Botanical Journal of the Linnean Society 55:1–33.

Vinson, J., and J.-M. Vinson. 1969. The saurian fauna of the Mascarene Islands. Mauritius Institute Bulletin 6:203–320.

Waser, N. M. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology 59:934–944.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443–466.

Natural History Editor: Henry M. Wilbur

# Appendix from D. M. Hansen et al., "Positive Indirect Interactions between Neighboring Plant Species via a Lizard Pollinator"

**(Am. Nat., vol. 169, no. 4, p. 000)**

## Additional Figures Illustrating Gecko Behavior and Floral Details

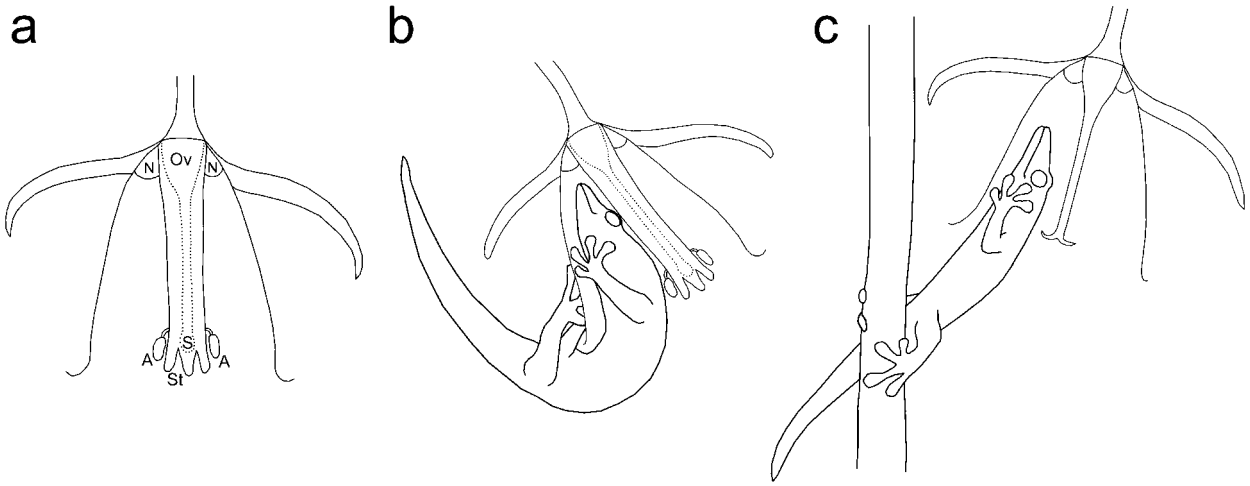



**Figure A1:** Schematic drawings of a *Trochetia blackburniana* flower and *Phelsuma cepediana* entering and pollinating the flowers. *a*, Longitudinal section of a flower: *Ov* = ovary, *N* = nectar drops, *S* = stigma, *A* = anthers, *St* = staminodes (cf. fig. A2). *b*, Male gecko inside a male-phase flower; this is a typical position of a gecko when climbing onto the flower from above (cf. fig. A4*b*). Pollen is deposited mostly on the upper back and neck of the gecko. *c*, Male gecko reaching into a female-phase flower (staminal tube has fallen off, and stigma has expanded) while holding onto a branch (cf. fig. A4*a*).

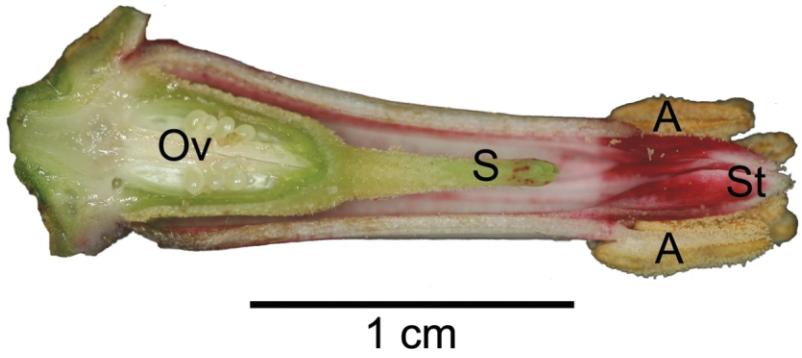



**Figure A2:** Longitudinal section of the staminal tube and gynoecium of an early male-phase *Trochetia blackburniana* flower. *Ov* = ovary, *S* = stigma, *A* = anthers, *St* = staminodes.

**Figure A3:** Close-up photo of a male-phase *Trochetia blackburniana* flower. The five narrowly overlapping staminodes at the end of the staminal tube prevent pollen from falling into the staminal tube and onto the stigma—which, in this phase, is still not receptive or even open (cf. fig. A2). Note the large amount of strikingly yellow nectar. Colored nectar has recently been shown to be a strong visual floral signal to *Phelsuma* geckos in Mauritius (Hansen et al. 2006).

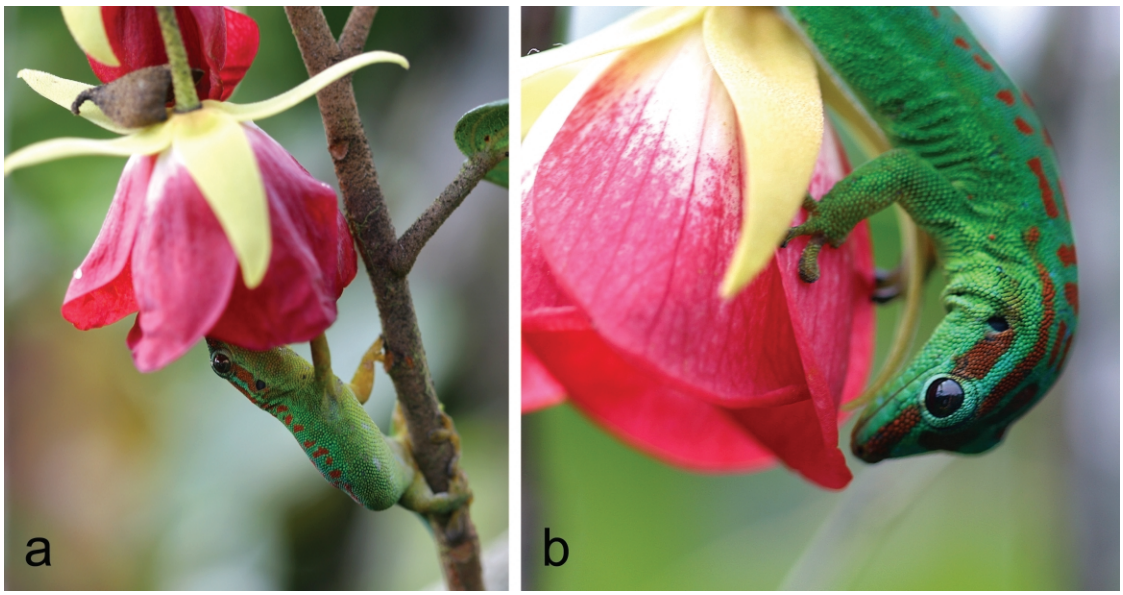



**Figure A4:** *Phelsuma cepediana* geckos approach the *Trochetia blackburniana* flowers in two different ways: they will try to reach out and grab hold of a flower while holding on to the branch (*a*) or, if this is not possible, they approach the flower from above, climbing down the peduncle, and enter the flower while hanging on to the petals or sepals.

# CHAPTER 4

## Habitat structure affects reproductive success of the rare endemic tree *Syzygium mamillatum* (Myrtaceae) in restored and unrestored sites in Mauritius

"A small herb of barely a finger's length growing on the tree trunks. It is so rare that, so far as is known, no one ever saw it before".

> –Swedish botanist Pehr Osbeck in 1757, giving the first scientific (mis-)description of the phenomenon now known as cauliflory

## ABSTRACT

Invasive alien plants affect the functioning of ecosystems by altering plant–animal interactions, such as pollination, which may impede natural regeneration of native plant species. In Mauritius, we studied the reproductive traits and pollination ecology of the rare endemic cauliflorous tree *Syzygium mamillatum* in a restored forest (all alien plant species removed) and an adjacent unrestored area (degraded by alien plants). Flowers of *S. mamillatum* were only visited by generalist bird species. Although the initial number of flower buds per tree in the restored forest tended to be higher than that of trees in the unrestored area, final fruit set and the number of seeds per fruit were lower in the restored forest. This corresponded with lower bird visitation rates in the restored area. Additionally, in budding stage most trees were severely attacked by lepidopteran larvae, and bud loss through herbivory was higher in the restored forest. Thus, the difference in reproductive performance of *S. mamillatum* between the two localities was caused by contrasting herbivorous attack and bird visitation behavior in restored and unrestored areas. Our findings illustrate the importance in restoration efforts of mimicking the original physical structure of habitats and interaction structure of interspecific relationships, and the difficulty of doing so given the imperfect knowledge and the reality that many native species have become locally extinct and replaced by exotic species.

## INTRODUCTION

Islands are often described as biodiversity hot-spots due to their relatively high levels of endemism and their disproportional contribution to global species diversity (Whittaker 1998, Myers *et al.* 2000). Invasive alien species, however, threaten the extant native biological diversity of island ecosystems (Cheke 1987, Simberloff 1995, Valido *et al.* 2002). Introduced species may interact with native species in several ways: many introduced plant species compete with natives for resources (Callaway & Aschehoug 2000, Daehler 2003) or benefit from mutualistic interactions with resident species, often to the detriment of native mutualisms (Bond 1994, Kearns *et al.* 1998, Simberloff & von Holle 1999, Richardson *et al.* 2000, Traveset & Richardson 2006). Although several studies have described the disruption of plant–animal interactions caused by competition for pollinators between alien and native plants (*e.g.*, Chittka & Schürkens 2001, Moragues & Traveset 2005), studies focusing on how mutualistic interactions are affected by a change in habitat structure associated with the spread of alien plant species are scarce. Native plant–animal interactions critical for plant reproduction can be disrupted by decreasing relative abundance or density of native plant populations (Ghazoul 2005, Ward & Johnson 2005) which consequently may alter pollinator behavior (Brown & Mitchell 2001, Ghazoul 2004). For example, Paton (2000) showed that the bird-pollinated plant *Astroloma conostephioides* native to Southern Australia was severely pollinator limited in degraded and invaded habitats due to the absence of migratory nectarivorous birds from these areas. In addition, introduced herbivores, which consume vegetative or reproductive parts of a plant, can exert negative pressures on the reproduction of native plants and/or on their pollinators (see Traveset & Richardson 2006 and references therein). Where populations are already compromised through habitat destruction, as is frequently the case on islands, such mechanisms might lead to localized extinction of plant or native pollinator populations.

The continuing decline of native plant and animal species in Mauritius, following the initial human-caused destruction of natural habitats, has been ascribed primarily to the spread of introduced species (Mauremootoo *et al.* in press-a), which now dominate the remaining upland forests (Vaughan & Wiehe 1941, Lorence & Sussman 1986, Cheke 1987). Today, only about two percent of Mauritius is covered with some degree of native forest (Page & d'Argent 1997), and even these remnants are severely degraded in most areas. To restore patches of native habitat in Mauritius, several Conservation Management Areas (CMAs) have been established since 1969. These restored sites are remnants of the

major original habitat types, and are fenced and regularly hand-weeded to eradicate introduced plant species. A survey in one of these restored areas (Brise Fér 'Old Plot', 1.26 ha), eight years after the start of restoration work in 1987, showed an improvement in natural regeneration of native flora compared to adjacent unrestored areas (Mauremootoo *et al.* in press-b). Thus, although there is evidence that the original plant community can regenerate following restoration, information on what actually limits regeneration of endemic plants in unrestored habitats is lacking. To improve current restoration strategies, it is necessary to understand the mechanistic basis of recruitment success of native plant species.

In addition to the direct impacts of invasive plants through competition for space, soil nutrients, light and water, the remaining populations of Mauritian plants are vulnerable to several threats. The original Mauritian fauna comprised a range of pollinating bird species, many of which are now locally or globally extinct. The loss of these native mutualists is thought to limit the natural regeneration of native plants that once were dependent on them (Kaiser 2006). The subsequent increasing abundance of introduced plants may have further disrupted native plant recruitment by modifying avian pollinator foraging behaviour as a consequence of the change in density and distance between neighbouring native plants. Removal of alien plants in restored areas may thus facilitate native plant recruitment through the recovery of associated pollination processes. Mauritius is therefore an ideal study system in which to explore the effects of such disruptions on the pollination of native plant species.

In this study, we investigated the effect of habitat restoration on pollination interactions and fruit/seed set of the rare endemic tree *Syzygium mamillatum* Bosser & Guého (Myrtaceae) in a restored and an adjacent unrestored, heavily invaded area. Because preliminary observations indicated that flower buds were being attacked by a herbivorous lepidopteran larvae, we also studied the impact of floral herbivory on the reproductive success of *S. mamillatum* in both areas.

## MATERIALS AND METHODS

### *STUDY AREA AND STUDY SPECIES*

We conducted the study in the Black River Gorges National Park in Mauritius between July 2003 and January 2004. Our study site was Brise Fér CMA (24 ha; 20°22′ S, 57°26′ E; 570–600 m asl), established by the National Parks and Conservation Service (NPCS) in 1996. The native tree community in the restored (CMA) and adjacent unrestored area of

Brise Fér consists mainly of canopy trees of the families Burseraceae, Ebenaceae, Celastraceae and Sapotaceae, and several sub-canopy trees of the genera *Gaertnera* (Rubiaceae) and *Syzygium* (Myrtaceae) (Strahm 1994). In the unrestored area, invasive plants, primarily guava *Psidium cattleianum* Sabine (Myrtaceae), privet *Ligustrum robustum* (Rox.) Blume (Oleaceae), and several weedy Melastomataceae, form an impenetrable understorey with a canopy of about 3–5 m in height. Native trees are found scattered within this site (see also Lorence & Sussman 1986). We surveyed approximately 60 ha in Brise Fér forest, of which the restored area covered 24 ha. The actual area in which the *Syzygium mamillatum* population occurred covered a total of 16 ha, approximately two thirds of which were in the restored area, and one third in the unrestored area. In total we found 120 mature trees of *S. mamillatum*. *Syzygium mamillatum* is endemic to Mauritius and the recorded individuals represent the last large extant population, accounting for 87 percent of all the mature trees of this species. The remaining 18 trees are either isolated individuals or small stands located within the boundaries of the National Park (M. Allet & J.-C. Sevathian, pers. comm.). Eighty-two mature healthy trees in the restored and 38 in the unrestored area were labeled, mapped, and included in our study. Natural regeneration of *S. mamillatum* is very limited. The only seedlings are found within 1 m of the maternal trees—suggesting a lack of seed-dispersal—and seedling survival is low close to maternal trees (D. M. Hansen, C. N. Kaiser & C. B. Müller, unpublished data). Since we worked with the only large extant population, replication across several sites was not possible. Nevertheless, we recognize the potential for within-site non-independence of the data and tested for spatial autocorrelation using Mantel tests (see below). Furthermore, we felt it was justified to investigate the effects of conservation interventions despite limited potential for site replication, precisely because *S. mamillatum* is endangered, but also because this species exemplifies the fate of many other endangered endemic tree species.

*Syzygium mamillatum* is a sub-canopy tree of 6–9 m in height, with a spectacular display of numerous, hermaphroditic flowers (calyx tube 8–10 mm long) on the trunk, located within 50 cm of the ground (Fig. 1a, b). The flowers contain nectar and are scentless. Each flower displays a hemispherical array of 80–90 anthers (filament length 8–10 mm), contains 15–20 ovules (Scott 1990), and produces a large and fleshy fruit. Preliminary experimental data suggested that *S. mamillatum* is largely self-incompatible (4% fruit set from 107 bagged flowers on seven plants; C.N. Kaiser unpublished data), as is the case for many arborescent Myrtaceae species (Lack & Kevan 1984, Beardsell *et al.*

1993, Proenca & Gibbs 1994). In 2003, *S. mamillatum* flowered from November to December.

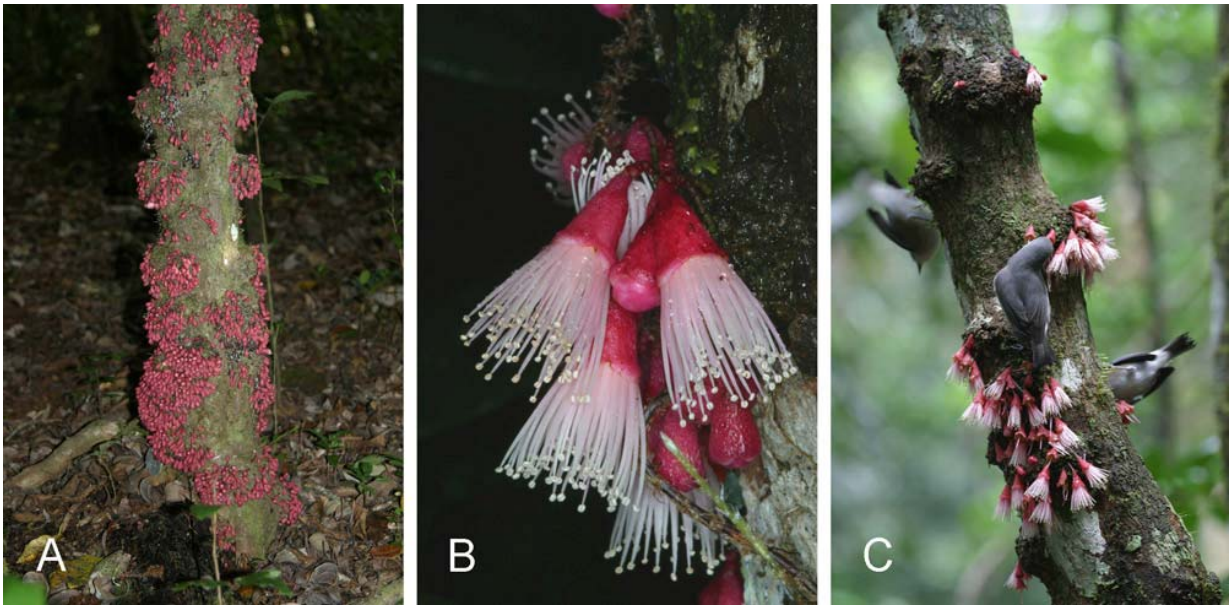



**FIGURE 1.** Floral characteristics of *Syzygium mamillatum*. (A) Buds are displayed on burrs along the stem, mainly at the base of the trunk. (B) Open flowers of *S. mamillatum* were visited by (C) Grey white-eyes foraging for nectar

*HABITAT STRUCTURE*

Before restoration, Brise Fér CMA was as degraded as the unrestored area (Strahm 1994) but since weeding began in 1996, native plant species have been slowly regenerating. As a result of restoration, the forest understorey in the restored area is now very open and little vegetation obstructs the floral display on tree trunks. In contrast, flowers on trees in the adjacent unrestored area are hidden from a human observer's view by a dense understorey of invasive plants. To investigate and quantify the differences in habitat structure, we measured tree density around the focal trees. We counted the number of native and exotic trees (exceeding a diameter at breast height of 3 cm) within a radius of 3 m around the *S. mamillatum* trees that were used for pollinator observations in the restored and the unrestored area ($N = 9$ in each area).

*HERBIVORY*

During flower bud development (October–November 2003) many *S. mamillatum* trees were attacked by the larvae of *Polyhymno* sp. (Lepidoptera, Gelechiidae, identified as an unknown species by Dr. Klaus Sattler, Natural History Museum London). Adult moths deposited their eggs in young flower buds and the developing larvae consumed the entire

bud over a period of approximately one week. We assessed the impact of these attacks by counting affected trees in the population and the number of affected buds for all trees in the population. The proportion of attacked buds was the number of destroyed buds divided by the initial total number of buds per tree. The spread of the herbivore through the tree population was surveyed weekly during the budding time.

*POLLINATOR OBSERVATIONS*

Between 12 November and 9 December 2003, pollinator observations were carried out on nine flowering trees in the restored and ten trees in the unrestored area in one-hour 'observation units' for a total of 15 h in each area. Each tree was observed for 1–3 h. Flowers did not open before 0700 h and anthesis lasted for one day only, and thus all observations were carried out between 0700 h and 1900 h. We spatially stratified observation trees across the population to cover the centre and the edges of the population evenly, and we selected those trees that had most flowers. Observations were carried out with binoculars (Leica 10 × 32) at a distance of larger than 6 m from the tree, which was considered far enough to avoid disturbing vertebrate flower visitors and sufficiently close to spot invertebrates. For each observed tree, we recorded the number of flowers observed, *i.e.*, visible on the trunk from our observation post (range: 18–450 flowers) and the total number of flowers displayed on the trunk. We recorded both visitation rate and bout length of flower visitors. Visitation rate is defined as the total number of visitors to the tree per hour divided by the number of visible flowers per tree. Bout length refers to the number of flowers probed per hour divided by the number of flowers observed.

To quantify the pollen load of avian flower visitors, we mistnetted birds for 12 h close to 12 trees in full blossom in the restored area. We caught a total of 25 birds, which were ringed before release to avoid re-sampling the same individuals. Pollen samples were collected by sweeping the forehead, breast and beak of each bird with a 25 $mm^2$ piece of basic fuchsin gel (Kearns & Inouye 1993). The sampled pollen grains were compared with a reference pollen collection (Kaiser 2006) and counted under a light microscope using a counting grid.

*PLANT MORPHOMETRICS AND REPRODUCTIVE PERFORMANCE*

For 119 *S. mamillatum* trees, we measured diameter at breast height (dbh), tree height (H) ($N$ = 112 trees), and mean distance to the nearest three conspecific neighbors (NND). We used Mantel statistics to investigate potential spatial autocorrelation for seed and fruit set

($N$ = 119 trees), and visitation rate ($N$ = 19 trees) (Sokal & Rohlf 1995). We calculated Euclidean distances as dissimilarity indices for seed set, fruit set and visitation rate, and compared observed correlation coefficients with the reference distribution of correlation coefficients based on 1000 randomized permutations (Legendre & Legendre 1998).

Early in the season before larvae attacked the developing buds, we counted emerging buds (see Fig. 1b) to quantify initial flower bud production of all trees in the population. Fruit set was calculated for each tree as the number of developing fruits divided by the number of buds that developed into open flowers. Fruit counts were carried out for each individual tree at the end of December 2003, approximately 10 days after the trees had finished flowering. A second fruit count was conducted mid February 2004 to investigate a potential reduction in fruit production that could be caused by maternal fruit abortion (Stephenson 1981), late-acting self-incompatibility (Proença & Gibbs 1994) or early inbreeding depression (Nic Lughadha 1998). The difference in number of fruits per tree between the two surveys divided by the initial number of developing fruits per tree is a measure of fruit loss. We collected a total of 1291 mature fruits (33% of total fruit crop) from 34 trees in the restored and 21 trees in the unrestored area and determined average fruit size (widest diameter), fruit weight, number of seeds, and seed weight.

*DATA ANALYSIS*

We used parametric tests when assumptions of normality could be met by transformation and applied appropriate non-parametric tests otherwise. We cube-root transformed visitation rates and bout lengths, and log-transformed plant morphometrics and reproductive parameters. Means across trees ± SE are given throughout unless otherwise noted.

To investigate the relationship between the amount of fruits lost between the two fruit counts and the initial number of fruits produced by each tree in both sites, we fitted a linear model with fruit loss as the response variable and the number of fruits per tree and site (restored/unrestored) as explanatory variables. Proportional data such as fruit set, bud and fruit loss were arcsine transformed to meet the assumptions of normality and homoscedasticity (Quinn & Keough 2002).

To predict the patterns of fruit set, seed set and bud loss in the restored and the unrestored site, we fitted three linear models. The response variables ‘number of seeds per fruit’, and ‘proportion of buds per tree destroyed by herbivores’ (bud loss) were fitted against the predictor variables ‘mean number of buds per tree’, ‘mean nearest neighbor

distance' (NND, both log-transformed), and site (restored/unrestored). To test whether pollinator visitation could predict fruit set, we replaced the predictor variable NND with 'visitation rate' in the third regression analysis. All analyses were conducted with the statistical package R.2.1.1 (R Development Core Team 2005).

## RESULTS

### *HABITAT STRUCTURE*

The density of native trees in the vicinity of *Syzygium mamillatum* was not significantly different between the restored and the unrestored area ($F_{1,16} = 0.278$, $P = 0.61$). However, the number of introduced trees within a 3 m radius of *S. mamillatum* trees in the unrestored area exceeded the number of native trees by a factor of six (restored: 18.7 ± 1.6 trees, unrestored: 109 ± 7.3 trees; $F_{1,16} = 187$, $P < 0.001$).

### *HERBIVORY*

The first attacks of buds by *Polyhymno* sp were recorded on 18 November 2003 (approximately two weeks prior to first anthesis) in the restored area on only three trees located within 10 m of each other. Three weeks later, approximately 50% of all trees were severely affected. Attacked trees lost on average 47.7 percent (± 3.5) of their buds, 60.5 percent (± 4.0) in the restored and 28.4 percent (± 5.7) in the unrestored site. There was a significant positive correlation between the number of buds per tree and the proportion of destroyed buds per tree ($r = 0.27$, $N = 69$, $P = 0.026$). Both the total number of buds destroyed by *Polyhymno* sp. and the proportion of buds destroyed per tree were higher in the restored than in the unrestored area (number of buds destroyed: $F_{1,67} = 18.9$, $P < 0.001$, proportion of buds destroyed: $F_{1,67} = 14.8$, $P < 0.001$). Overall, the higher number of buds destroyed per tree in the restored area could be explained by management scheme, 'mean number of flowers' per tree and 'nearest neighbor distance' ($F_{3,65} = 5.93$, $P = 0.001$).

### *POLLINATOR OBSERVATIONS*

We observed a total of 89 interactions between flowering trees of *S. mamillatum* and three species of bird (Table 1). The endemic grey white-eye *Zosterops mauritianus* Gmelin (Zosteropidae) (Fig. 1c) was the most abundant flower visitor of *S. mamillatum* in the restored and the unrestored forest, but we observed fewer individual birds in the unrestored area (Table 1). The number of individuals of the introduced red-whiskered bulbul *Pycnonotus jocosus* L. (Pycnonotidae) was small and similar in both areas. The

endemic Mauritius bulbul *Hypsipetes olivaceus* Jardine & Selby (Pycnonotidae) was only observed at trees in the restored area. Pollen swabs taken from one mist-netted Madagascar fody *Foudia madagascariensis* L. (Passeridae) revealed that this species may be a pollen vector for *S. mamillatum* although it was not observed visiting flowers of *S. mamillatum*. Pollen from *S. mamillatum* was indistinguishable from pollen of other species in the genus using light microscopy. Thus, we could not assume that every pollen grain counted belonged to *S. mamillatum*. However, despite an extensive search we found no other flowering *Syzygium* species within our study area during the time of mist-netting. Only a few pollen grains from other plant families were found in the samples, which suggested that birds visited mainly *S. mamillatum*.

In contrast to our prediction, the overall mean visitation rate (*i.e.*, the number of visitors per tree divided by the number of flowers observed) was lower in the restored than in the unrestored area ($t = -2.65$, df = 17, $P = 0.017$; Fig. 2a). Although mean bout lengths was not statistically different in both habitats ($t = -1.67$, df = 17, $P = 0.12$), birds in the unrestored area probed almost twice as many flowers per visit than those in restored areas (Fig. 2b). No significant spatial autocorrelation was found for visitation rate ($r = -0.12$, $P = 0.72$), hence our sampled trees can be considered as statistically independent within-site replicates. Trees with many flowers attracted more birds than trees with fewer flowers ($r = 0.48$, $N = 19$, $P = 0.036$), and birds that visited trees with many flowers probed, on average, more flowers than those on trees with fewer flowers ($r = 0.59$, $N = 19$, $P = 0.004$). Visitation rate of the grey white-eye was higher in the unrestored than in the restored area although the total number of grey white-eyes observed was twice as high in the restored area (Table 1). There was no significant difference in visitation rate of red-whiskered bulbuls between sites. The grey white-eye, despite being the most abundant flower visitor, carried significantly fewer *Syzygium* pollen grains than the red-whiskered bulbul, the second most abundant visitor (Table 1).

### *Plant morphometrics and reproductive performance*

Trees in the restored and the unrestored area were of similar height ($F_{1,110} = 0.02$, $P = 0.90$), although trees at the restored site had a larger diameter at breast height (dbh; 6.5 ± 0.19 cm) than trees in the unrestored area (5.6 ± 0.18 cm; $F_{1,116} = 4.58$, $P = 0.035$). We found a positive relationship between dbh and the number of buds per tree ($r = 0.37$, $N = 118$, $P < 0.001$), indicating that bigger trees were more abundant in the restored area and produced more buds.

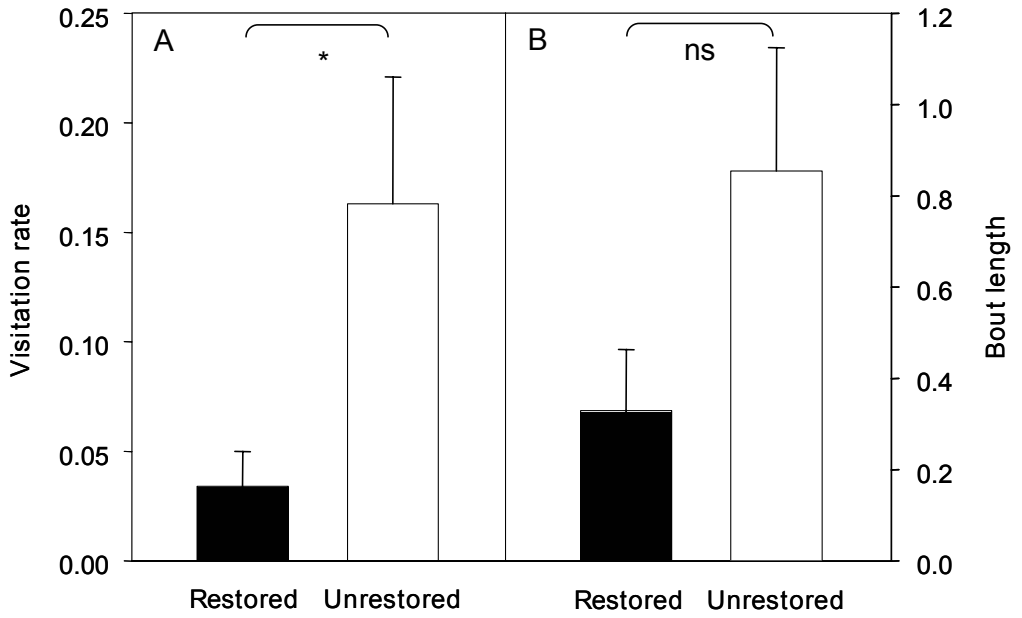



**FIGURE 2.** Differences in mean (+ SE) (A) visitation rate and (B) bout length per tree in restored ($N = 9$ trees) and unrestored areas ($N = 10$ trees). *, $P < 0.05$, ns, not significant. Visitation rate is the number of birds visiting during one observation unit (= per tree) divided by the number of observed flowers. Bout length refers to the number of probed flowers per tree per observation unit divided by the number of observed flowers.

Several trees in the restored site produced a high number of buds (14.6% > 2000 buds, 691 ± 1295 SD) in contrast to trees in the unrestored area (3.1% > 2000 buds, 338 ± 597 SD). However, we found no statistically significant differences in mean number of buds and flowers per tree between the two areas presumably due to a greater variance in the restored area (Fig. 3a). Similarly, mean fruit production per tree was not significantly higher in the restored than in the unrestored area (Fig. 3b). Since the majority of *S. mamillatum* trees grow in the restored area, we recorded an overall 4.5-fold difference in total number of fruits (8343 vs. 1816) and a 3.5-fold difference in the total number of seeds (18,772 vs. 4907) between the restored and the unrestored area. During the second fruit count in February 2004, we counted a total of 2744 fruits in the restored and 974 fruits in the unrestored site. The number of fruits per tree was not related to the proportion of fruits lost (only trees with > 10 fruits; $F_{1,63} = 0.93$, $P = 0.34$), and the latter did not differ between sites ($F_{1,63} = 1.39$, $P = 0.24$).

Average fruit set per tree was lower in the restored than in the unrestored area (overall fruit set: 0.248 ± 0.019), and mean number of seeds per fruit (overall 2.14 ± 0.39) showed a trend ($P = 0.057$) towards fewer seeds per fruit in the restored area (Fig. 3c). Neither fruit nor seed set data were spatially autocorrelated (fruits: $r = –0.05$, $P = 0.92$;

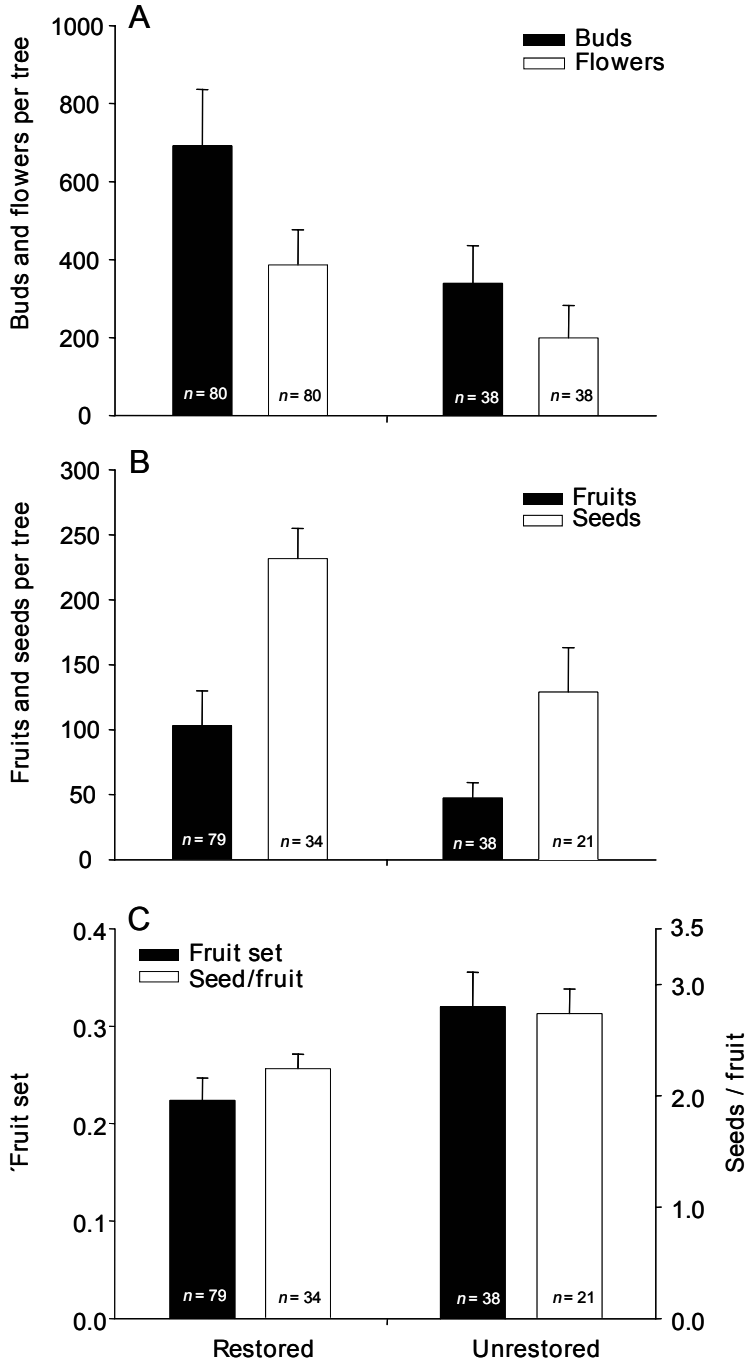



**FIGURE 3.** Differences in mean (+ SE) (A) number of buds (initial) and flowers (after herbivore attack) per tree, (B) number of fruits and total number of seeds/fruit per tree, and (C) fruit set and number of seeds/fruit per tree in restored and unrestored areas. Fruit set is defined as number of fruits divided by number of flowers per tree. Total number of seeds per fruit was calculated for each tree based either on mean seeds/fruit from individual counts of trees or from the overall mean. In (A) both number of buds and flowers per tree were not significantly different between management schemes (buds: $t = -1.21$, df = 117, $P = 0.23$, flowers: $t = -0.18$, df = 117, $P = 0.86$). (B) Number of fruits ($t = 0.58$, df = 115, $P = 0.57$) and seed/fruit per tree ($t = 1.18$, df = 115, $P = 0.24$) were not different between sites. In (C) fruit set was significantly different between sites ($t = -2.72$, df = 115, $P = 0.007$) and number of seeds per fruit per tree showed a trend towards more seeds in fruits from the unrestored area than fruits from the restored area ($t = -1.95$, df = 53, $P = 0.056$). For the analysis of number of seeds per fruits, fruits were collected from 55 trees in total.

seeds: $r = 0.03$, $P = 0.26$). Fruit set increased significantly with increasing visitation rate ($r = 0.53$, $N = 19$, $P = 0.020$). Fruit set and the number of seeds per fruit could not be sufficiently explained by management scheme, 'mean number of flowers' per tree and 'visitation rate' (fruit set: adj. $R^2 = 0.23$, $F_{3,14} = 2.68$, $P = 0.087$) or nearest neighbor distance, respectively (seeds/fruit: adj. $R^2 = 0.05$, $F_{3,51} = 1.86$, $P = 0.15$).

Mean fruit size per tree in the restored area was 17.4 ± 0.32 mm, while it was larger in the unrestored areas, measuring 18.4 ± 0.33 mm ($F_{1,53} = 4.57$, $P = 0.037$). Although there was a strong positive correlation between fruit size and weight ($r = 0.86$, $P < 0.001$), we found no significant difference in fruit weight between sites (restored: 5.60 ± 0.28 g, unrestored: 6.25 ± 0.33 g, $F_{1,53} = 2.18$, $P = 0.146$). Interestingly, 73 percent of fruits overall developed at a height of less than 30 cm on the trunk.

## DISCUSSION

### *HERBIVORY LIMITS FRUIT PRODUCTION*

A substantial proportion (48%) of *S. mamillatum* buds in the population was destroyed by *Polyhymno* larvae. Little is known about the genus *Polyhymno* and host–plant records for only four *Polyhymno* species exist for Fabaceae in the Nearctic, India and the African tropics (Robinson *et al.* 2001). Given the natural distribution of other Polyhymno species and the high attack rate on *S. mamillatum*, *Polyhymno* sp. may be introduced to Mauritius. The rate of bud destruction was higher inside the restored area than outside (~61% vs. ~28%), which may be explained by lepidopteran herbivores relying primarily on visual and olfactory cues for host–plant selection (Finch & Collier 2000). Host trees in the restored area may simply be more visible or smell more conspicuously than host trees in the unrestored area, which are obscured by the dense undergrowth (Wiklund 1984). A trade-off between maximizing floral display to attract pollinators and minimizing visibility to herbivores has been stressed by other authors (*e.g.*, Fenner *et al.* 2002, Juenger *et al.* 2005) and may play a role in our system. The positive correlation between the attack rates and number of buds per tree suggests positive density-dependence. The negative impact of herbivorous larvae on the reproduction of endangered endemic trees in Mauritius can be substantial and warrants further research.

### *POLLINATION BIOLOGY*

The endemic grey white-eye was the most abundant visitor to *S. mamillatum* flowers, followed by the endemic Mauritius bulbul and the introduced red-whiskered bulbul. Bird

pollination of *S. mamillatum* fits in well with the overall generalized pollination system of the Myrtaceae with a wide range of vertebrate and invertebrate floral visitors (Hopper 1980, Lack & Kevan 1984, Hingston *et al.* 2004, Boulter *et al.* 2005). Nocturnal flower visitors, such as hawkmoths, cannot be excluded with certainty, but their contribution to pollination is likely to be minimal because flowers opened early in the morning and anthesis lasted for one day only.

Total fruit and seed production and pollinator abundance were greater in the restored area. However, visitation rate (*i.e.*, number of visits per flower) was higher in the unrestored area, resulting in higher fruit set and a trend towards more seeds per fruit, and fruits were larger but not heavier in the unrestored area. Thus, why do trees, with respect to pollination, appear to perform better in the unrestored area? Gross fruit and seed production is largely dependent on the number of trees in each area. There are at least two possible explanations for the discrepancy in visitation rates and fruit-to-flower ratios between sites.

First, avian foraging behavior may differ with habitat structure. Trees surrounded by dense undergrowth may offer better protection from predators, resulting in longer stays and potentially greater pollen transfer in such patches. Bird visitation behavior may also explain differences in seed set. We showed a positive correlation between floral abundance and bout length, which could have resulted in a higher proportion of intra-tree pollen transfer in the restored area and consequently in lower seed set due to self-incompatibility. Klinkhamer and de Jong (1993) proposed that optimal plant fitness is obtained by displaying an intermediate number of flowers. Trees in the restored area which bear many flowers may therefore experience more self-pollination, resulting in lower fruit set and number of seeds per fruit than trees in the unrestored habitat, where floral abundance was low. Proença and Gibbs (1994) described late-acting self-incompatibility for several Brazilian Myrtaceae species, which might also be the case for *S. mamillatum*. Our study showed that the flower-to-fruit ratio in *S. mamillatum* decreased between December 2003 and February 2004, particularly in the restored site (see also Nic Lughadha 1998).

Second, hermaphroditic flowering plants often produce an initial excess of flowers that does not contribute to female fitness through fruit or seed production (Sutherland 1987). Several different mechanisms have been proposed to explain how floral excess production elevates female fitness (Burd 1998). Larger floral displays may attract more pollinators (Conner & Rush 1996), may allow higher fruit set in resource-rich years and

thereby increase lifetime fitness (Lloyd 1980), or may provide a 'reproductive assurance' against losses to, for example, herbivores or fungi (Ehrlén 1991, Hingston & Potts 2005). Another female function mechanism is selective abortion of fruits. Floral overproduction provides a larger pool from which higher quality fruits can be selectively matured (Janzen 1977, Stephenson 1981), provided there is variation in quality, such as in number of seeds per fruit (*e.g.*, Waser *et al.* 1995) or paternity patterns (Charnov 1979, Niesenbaum 1999). In *S. mamillatum* trees in the restored area, these mechanisms may act in concert or individually. Overproduction and fruit maturation is likely to be dependent on these effects and they appear to be stronger in the restored area.

To summarize, subtle changes in bird behavior through habitat structure could explain higher reproductive performance of trees in the unrestored area. It is, however, encouraging that fruit and seed production in the restored area is high, which is the first requirement for habitat restoration to be successful. Even if the unrestored, relatively dense area may be better for pollinators, it is likely that high competition for nutrients and light has a negative effect on seedling recruitment and reproductive success was actually higher in the restored area. At population level, successful conservation of *S. mamillatum* is therefore closely linked to the removal of invasive plants from relatively small confined areas.

### *POLLINATOR IDENTITY*

Since specialized nectar-feeding birds are locally extinct in Brise Fér, the pollination service to *S. mamillatum* must be fulfilled by generalist extant native and introduced bird species. The grey white-eyes was the most numerous visitor, but it may not be the most efficient pollinator of *S. mamillatum*. Despite having a shorter beak and, therefore, having to probe flowers of *S. mamillatum* more deeply to obtain nectar, the grey white-eye carried only half as many pollen grains as the red-whiskered bulbul. When the red-whiskered bulbul forages for nectar, the prominent feather-crest often touches the anthers. Therefore, the red-whiskered bulbul is most likely the more efficient pollinator of *S. mamillatum*, but its role as a major seed disperser of invasive plant species may overall hamper habitat restoration (Linnebjerg 2006). The large amount of *S. mamillatum* pollen found on the introduced Madagascar fody is intriguing: owing to its short beak, this species must probe the flowers deeply to reach the nectar. This may suggest that the locally extinct Mauritian fody *Foudia rubra* (Gmelin) was once an efficient pollinator of *S. mamillatum*.

*PLANT RECRUITMENT*

Successful and sustainable reproduction of plant populations depends firstly on intact plant–pollinator interactions and subsequently on seed-dispersal and seedling recruitment. Physiological and evolutionary theories have been proposed to explain the occurrence of cauliflory and caulicarpy (fruits on the trunk) (Haberlandt 1893, cited in Richards 1996), but few studies have focused on their ecological significance. It may be that caulicarpy, rather than being seen simply as the inevitable consequence of cauliflory, is the more significant trait with respect to selective forces acting on the reproductive display of *S. mamillatum* (see also van der Pijl 1957). As a result, this peculiar flower presentation low on the stem may occur primarily to ease access for ground-dwelling seed dispersers to the fruits (Hopper 1980, Beardsell *et al.* 1993, Warren *et al.* 1997, Kaiser 2006). Likely ground-dwelling candidates among the many extinct frugivores in Mauritius are the two giant tortoise species, *Cylindraspis triserrata* and *C. inepta*, or the giant skink *Leiolopisma mauritiana*. The absence of seed dispersers may explain the lack of natural regeneration of *S. mamillatum* in Brise Fér. Indeed, in another study, we show how extant giant Aldabra tortoises can be used as ecological analogue seed dispersers of *S. mamillatum* (Chapter 5),

*IMPLICATIONS FOR CONSERVATION*

Although the degradation of the native Mauritian flora and the subsequent restoration programs have been well described (Vaughan & Wiehe 1941, Page & d'Argent 1997, Mauremootoo *et al.* in press-b), little is known about the rate of regeneration in restored areas (CMAs) and, therefore, the success of these management strategies.

Our finding that trees in unrestored area perform slightly better overall, presumably due to the denser understorey, indicates that the current management strategy—at least in the short term—may be missing a crucial factor. Paradoxically, the restoration process of weeding exotic plants creates a disturbance that may affect the behavior of invertebrate pest insects and bird pollinators. As a practical recommendation, we suggest that weeding in restored areas should be conducted with a minimum of disturbance and perhaps as a temporally more gradual removal of exotic plants, which would promote structural habitat heterogeneity (see Hobbs & Huenneke 1992, D'Antonio & Meyerson 2002).

In conclusion, for the management of rare declining species it is important to acknowledge mutualistic and antagonistic interactions and to consider that these interactions may be ‘provided’ by exotic and/or generalist species since formerly

widespread specialists or generalists are now locally extinct. Our study highlights the futility in reconstructing original habitat conditions without incorporating information on ecosystem functions. On tropical oceanic islands, little is known about the original network of interactions among native plant and animal species. Although it will be impossible to consider the full community background, restoration should focus at least on the functionally most important interactions and on structural habitat traits, taking steps towards a broader understanding of ecosystem functioning.

## ACKNOWLEDGMENTS

We are grateful to the Mauritian Wildlife Foundation (MWF) and the National Parks and Conservation Services (NPCS) of Mauritius for logistic support and the permission to work in Brise Fér. J. Mauremootoo, M. Allet, R. Jumoorty and J.-C. Sevathian gave valuable advice on the target plant and in the field. We thank L. Reiter, K. Edmunds, and K. Henson for their help in the field and I. Hove Sørensen and C. Jensen Marcet for help with the mist netting. J. Nüscheler helped with pollen counts. J. Krauss, S. Härri, M. Kraemer, J. M. Olesen, J. Ghazoul, N. Waser and N. Bunbury provided helpful suggestions on earlier drafts. The project was funded by the Swiss National Science Foundation (grant no. 631-065950 to CBM) and the Roche Research Foundation.

## REFERENCES

BEARDSELL, D. V., S. P. OBRIEN, E. G. WILLIAMS, R. B. KNOX, and D. M. CALDER 1993. Reproductive-biology of Australian Myrtaceae. Australian Journal of Botany 41(4-5): 511-526.

BOND, W. J. 1994. Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 344(1307): 83-90.

BOSSER, T. C. J., T. CADET, J. GUEHO, and W. MARAIS 1983. Flore des Mascareignes - La Réunion, Maurice, Rodrigues. The Mauritian Sugar Industry Research Institute, Port Louis.

BOULTER, S. L., R. L. KITCHING, B. G. HOWLETT, and K. GOODALL 2005. Any which way will do—the pollination biology of a northern Australian rainforest canopy tree (*Syzygium sayeri*, Myrtaceae). Botanical Journal of the Linnean Society 149(1): 69-84.

BROWN, B. J., and R. J. MITCHELL 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129: 43-49.

BURD, M. 1998. 'Excess' flower production and selective fruit abortion: a model of potential benefits. Ecology 79(6): 2123-2132.

CALLAWAY, R. M., and E. T. ASCHEHOUG 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290(5499): 2075-2075.

CHARNOV, E. L. 1979. Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Sciences of the United States of America 76(5): 2480-2484.

CHEKE, A. S. 1987. The ecology of the smaller land birds of Mauritius. *In* A. W. Diamond (Ed.). Studies of Mascarene Island birds, pp. 151-207. Cambridge University Press, Cambridge.

CHITTKA, L., and S. SCHÜRKENS 2001. Successful invasion of a floral market. Nature 411: 653.

CONNER, J. K., and S. RUSH 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. Oecologia 105(4): 509-516.

D'ANTONIO, C. M., and L. A. MEYERSON 2002. Exotic plant species as problems and solutions in ecological restoration: a synthesis. Restoration Ecology 10(4): 703-713.

DAEHLER, C. C. 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology, Evolution, and Systematics 34(1): 183-211.

EHRLÉN, J. 1991. Why do plants produce surplus flowers? – A reserve-ovary model. American Naturalist 138(4): 918-933.

ENDRESS, P. K. 1994. Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

FENNER, M., J. E. CRESSWELL, R. A. HURLEY, and T. BALDWIN 2002. Relationship between capitulum size and pre-dispersal seed predation by insect larvae in common Asteraceae. Oecologia 130(1): 72-77.

FINCH, S., and R. H. COLLIER 2000. Host-plant selection by insects – a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis Et Applicata 96(2): 91-102.

GHAZOUL, J. 2004. Alien abduction: Disruption of native plant-pollinator interactions by invasive species. Biotropica 36(2): 156-164.

GHAZOUL, J. 2005. Pollen and seed dispersal among dispersed plants. Biological Reviews 80(3): 413-443.

HINGSTON, A. B., and B. M. POTTS 2005. Pollinator activity can explain variation in outcrossing rates within individual trees. Austral Ecology 30(3): 319-324.

HINGSTON, A. B., B. M. POTTS, and P. B. MCQUILLAN 2004. Pollination services provided by various size classes of flower visitors to *Eucalyptus globulus* ssp. *globulus* (Myrtaceae). Australian Journal of Botany 52(3): 353-369.

HOBBS, R. J., and L. F. HUENNEKE 1992. Disturbance, diversity, and invasion – implications for conservations. Conservation Biology 6(3): 324-337.

HOPPER, S. D. 1980. Pollination of the rain-forest tree *Syzygium tierneyanum* (Myrtaceae) at Kuranda, Northern Queensland. Australian Journal of Botany 28(2): 223-237.

JANZEN, D. H. 1977. Note on optimal mate selection by plants. American Naturalist 111(978): 365-371.

JUENGER, T., T. C. MORTON, R. E. MILLER, and J. BERGELSON 2005. Scarlet gilia resistance to insect herbivory: the effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. Evolutionary Ecology 19(1): 79-101.

KAISER, C. N. 2006. Functional integrity of plant-pollinator communities in restored habitats in Mauritius. PhD Dissertation. University of Zurich, Zurich, Switzerland.

KEARNS, C. A., and D. W. INOUYE 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.

KLINKHAMER, P. G. L., and T. J. DE JONG 1993. Attractiveness to pollinators - a plants dilemma. Oikos 66(1): 180-184.

LACK, A. J., and P. G. KEVAN 1984. On the reproductive biology of a canopy tree, *Syzygium syzygoides* (Myrtaceae), in a rain forest in Sulawesi, Indonesia. Biotropica 16(1): 31-36.

LEGENDRE, P., and L. LEGENDRE 1998. Numerical ecology. Elsevier Science, Amsterdam.

LINNEBJERG, J. F. 2006. The ecological impact of the invasive Red-whiskered bulbul *Pycnonotus jocosus* in Mauritius. MSc thesis. University of Aarhus, Aarhus, Denmark.

LLOYD, D. G. 1980. Sexual strategies in plants 1. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytologist 86(1): 69-79.

LORENCE, D. H., and R. W. SUSSMAN 1986. Exotic species invasion into Mauritius wet forest remnants. Journal of Tropical Ecology 2: 147-162.

MAUREMOOTOO, J. R., I. WATT, and F. B. V. FLORENS in press-a. Mauritius biodiversity. *In* V. Bachraz and R. Atkinson (Eds.). The biodiversity hotspot of Mauritius, past, present and future. National Parks and Conservation Service Mauritius, Reduit.

MAUREMOOTOO, J. R., I. WATT, and C. V. TOWNER-MAUREMOOTOO in press-b. Mauritius institutional conservation capacity. *In* V. Bachraz and R. Atkinson (Eds.). The biodiversity hotspot of Mauritius, past, present and future. National Parks and Conservation Service Mauritius, Reduit.

MORAGUES, E., and A. TRAVESET 2005. Effects of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. Biological Conservation 122: 611-619.

MYERS, N., R. A. MITTERMEIER, C. G. MITTERMEIER, G. A. B. DA FONSECA, and J. KENT 2000. Biodiversity hotspots for conservation priorities. Nature 403(6772): 853-858.

NIC LUGHADHA, E. 1998. Preferrential outcrossing in *Gomedesia* (Myrtaceae) is maintained by a post-zygotic mechanism. *In* S. J. Owens and P. J. Rudall (Eds.). Reproductive biology in systematics, conservation and economic botany, pp. 363-379. Royal Botanical Gardens, Kew, London.

NIESENBAUM, R. A. 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). American Journal of Botany 86: 261-268.

NOVOTNY, V., and Y. BASSET 2005. Host specificity of insect herbivores in tropical forests. Proceedings of the Royal Society B-Biological Sciences 272: 1083-1090.

PAGE, W. S., and G. D'ARGENT 1997. A vegetation survey of Mauritius. Mauritian Wildlife Foundation, Port Louis, Mauritius.

PATON, D. C. 2000. Disruption of bird-plant pollination systems in southern Australia. Conserv. Biol. 14: 1232-1234.

PROENCA, C. E. B., and P. E. GIBBS 1994. Reproductive biology of eight sympatric Myrtaceae from Central Brazil. New Phytologist 126: 343-354.

QUINN, G. P., and M. J. KEOUGH 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

R DEVELOPMENT CORE TEAM 2005. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RICHARDS, P. W. 1996. The tropical rain forest: an ecological study. Cambridge University Press, Cambridge, UK.

RICHARDSON, D. M., N. ALLSOPP, C. M. D'ANTONIO, S. J. MILTON, and M. REJMÁNEK 2000. Plant invasions - the role of mutualisms. Biological Review 75: 65-93.

ROBINSON, G. S., P. R. ACKERY, I. J. KITCHING, G. W. BECCALONI, and L. M. HERNÁNDEZ 2001. Hostplants of the moth and butterfly caterpillars of the Oriental Region. Southdene, Kuala Lumpur.

SCOTT, A. J. 1990. Myrtacées. *In* T. C. J. Bosser, T. H. Cadet, J. Guého and W. Marais (Eds.). Flore des Mascareignes, pp. 1-70. The Mauritius Sugar Industry Research Institute, Réduit.

SIMBERLOFF, D. 1995. Habitat fragmentation and population extinction of birds. Ibis 137 (suppl. 1): s105-s111.

SIMBERLOFF, D., and B. VON HOLLE 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological Invasions 1(21-32): 21-32.

SOKAL, R. R., and F. J. ROHLF 1995. Biometry. W. H. Freeman and Company, New York, New York.

STEPHENSON, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12(1): 253-279.

STRAHM, W. 1994. The conservation and restoration of the flora of Mauritius and Rodrigues. PhD Dissertation. University of Reading, Reading, UK.

SUTHERLAND, S. 1987. Why hermaphroditic plants produce many more flowers than fruits - experimental tests with *Agave mckelveyana*. Evolution 41(4): 750-759.

TRAVESET, A., and D. M. RICHARDSON 2006. Biological invasions as disruptors of plant reproductive mutualisms. Trends in Ecology & Evolution 21: 208-216.

VALIDO, A., Y. L. DUPONT, and D. M. HANSEN 2002. Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. Acta Oecologica 23(6): 413-419.

VAN DER PIJL, L. 1957. The dispersal of plants by bats (Chiropterochory). Acta Botanica Neerlandica 6: 291-315.

VAUGHAN, R. E., and P. O. WIEHE 1941. Studies on the vegetation of Mauritius: III. The structure and development of the upland climax forest. Journal of Ecology 29: 127-160.

WARD, M., and S. D. JOHNSON 2005. Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). Oikos 108(2): 253-262.

WARREN, J. M., D. Z. EMAMDIE, and KALAI 1997. Reproductive allocation and pollinator distributions in cauliflorus trees in Trinidad. Journal of Tropical Ecology 13: 337-345.

WASER, N. M., R. G. SHAW, and M. V. PRICE 1995. Seed set and seed mass in *Ipomopsis aggregata* - variance partitioning and inferences about postpollination selection. Evolution 49(1): 80-88.

WHITTAKER, R. J. 1998. Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford, UK.

WIKLUND, C. 1984. Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia 63(1): 23-29.

# CHAPTER 5

**Endangered endemic plants on tropical oceanic islands: seed dispersal, seedling establishment, and ecological analogues**

"If ... ecologists and evolutionary biologists wish to determine who eats fruit, who carries sticky seeds, and who browses, grazes, tramples, and voids that segment of the habitat that would have been within reach of a variety of megafaunal trunks, tusks, snouts, tongues and teeth, the missing megafauna must be considered."

–Janzen & Martin, 1982

## ABSTRACT

The Janzen-Connell model states that host-specific natural enemies may have a disproportionately large negative effect on progeny close to maternal trees. The vast majority of both experimental and theoretical studies addressing the Janzen-Connell model have explored how it can explain existing patterns of species diversity in tropical mainland areas. We provide the first experimental investigation of the predictions of the Janzen-Connell model on an oceanic island, in a conservation context. In Mauritius we studied seed germination and seedling survival patterns of the critically endangered endemic plant *Syzygium mamillatum* (Myrtaceae) in relation to proximity to maternal trees. We also experimentally evaluated the use of ecological analogue animals to resurrect the functional component of extinct frugivores. We successfully used giant Aldabran tortoises as ecological analogues for extinct Mauritian frugivores. Seed germination patterns were strongly affected by propagule type (whole fruits, depulped seeds, and gut-passed seeds), but there was no effect of proximity to maternal tree on germination patterns. Seedlings close to maternal trees had fewer leaves, suffered more damage from natural enemies, and survived less well compared to seedlings away from maternal trees. Effects of tortoise gut-passage were negative at the seed germination stage, but seedlings from gut-passed seeds grew taller, had more leaves, and suffered less damage from natural enemies than any of the other seedlings. The observed strong negative effects of proximity to maternal tree on seedlings provides the first experimental evidence of a distance-dependent Janzen-Connell effect on an oceanic island. In contrast to recent controversy about the use of non-indigenous extant megafauna in North America and elsewhere, we argue that Mauritius and other oceanic islands are ideal study systems in which to empirically explore the use of ecological analogue species in restoration ecology.

## INTRODUCTION

Animal-mediated seed dispersal and subsequent differences in seedling establishment and survival in relation to distance from adult conspecific plants are important factors in the dynamics of tropical forests (Harms *et al.* 2000; Howe & Miriti 2000). This has been intensely studied during the last three decades in the framework of the Janzen-Connell model (Janzen 1970; Connell 1971; Clark & Clark 1984), which states that host-specific seed predators, or seedling herbivores and pathogens may have a disproportionately large negative effect on progeny close to maternal trees. Hence, a major positive effect of seed dispersal away from maternal trees is that seeds and seedlings may escape from adverse pressures by natural enemies into a more benign neighbourhood for establishment and survival (Howe & Smallwood 1982). However, the vast majority of both experimental and theoretical studies addressing the Janzen-Connell model have been primarily concerned with exploring how it can explain existing patterns of species diversity and were less concerned with the potential importance of this pattern for conservation ecology (reviewed in e.g. Wright 2002).

In our study we experimentally address how predictions of the Janzen-Connell model may be important in a conservation context on tropical islands, where endangered plants are often found in very low numbers within small areas. Furthermore, we assess the use of ecological analogue seed-dispersing animal species to resurrect the functional component of extinct endemic frugivores.

### *THE JANZEN-CONNELL MODEL ON OCEANIC ISLANDS*

Only few studies have investigated aspects of seed dispersal and seed- and seedling survival in the framework of the the Janzen-Connell model on oceanic islands (Galápagos, Clark & Clark 1981; other Pacific islands, Lee 1985, Wiles *et al.* 1987; Canary Islands, Arevalo & Fernandez-Palacios 2003). Most of these studies show that saplings and juvenile trees are mostly found away from adult trees; however, in the Canary Islands Arevalo and Fernandez-Palacios (2003) found no effect of distance to conspecific adults on sapling density, suggesting that Janzen-Connell spacing plays a minor role in this forest.

Despite a long scientific history of using islands as natural laboratories for ecological and evolutionary studies (e.g. Vitousek *et al.* 1995; Grant 1998), we are not aware of any studies that have experimentally investigated seed germination and the fate of post-germination seedlings in the framework of the Janzen-Connell model on oceanic

islands. Consequently, while it is acknowledged that oceanic islands harbour many of the most critically endangered plant species in the world, we know next to nothing about how one of the most widely studied ecological patterns affects the regeneration and longer-term survival of these plants.

Two key points emerge in relation to the Janzen-Connell model and how it applies to the ecology and conservation of plants in oceanic island ecosystems. Firstly, patterns of seed- and seedling mortality on islands may be different from those found in mainland ecosystems. Generally, a high host-specificity of herbivores and pathogens is an assumption of the Janzen-Connell model of seedlings and sapling distribution (Clark & Clark 1984). Island ecosystems are often simpler than mainland ecosystems, in which case we would expect more generalist than specialist seed predators and herbivores on islands than on the mainland. If so, we could expect Janzen-Connell patterns to be less prevalent on oceanic islands than in comparable mainland habitats. There have been many studies on specialist versus generalist herbivorous insects in tropical forests (reviewed in Novotny & Basset 2005), but very little is known about the relative importance of specialist and generalist insect herbivores on oceanic islands (Ribeiro *et al.* 2005). The second key point is that studies of Janzen-Connell patterns are more urgent in relation to conservation management for oceanic islands than for most mainland ecosystems. Pristine oceanic islands typically harboured fewer species of frugivorous vertebrate than comparable mainland areas, and many of those few species are now extinct (Cox *et al.* 1991). Thus, there are currently many oceanic islands where the frugivorous members of the afterlife (*sensu* Lawton 1995) outnumber the living, and many seed dispersal interactions are likely to have been lost. Today, the remaining native and endemic flora and fauna of many oceanic islands are often crammed into much smaller remnant patches of native habitats than those on the mainland. Therefore, if Janzen-Connell patterns are indeed prominent on oceanic islands, endangered plant species relying on vertebrate dispersal by now-extinct animals face a double peril: not only do they lack most of the agents that once mediated the escape and establishment of their progeny away from maternal trees, but present-day native habitats may be too small to support viable populations of plants that exhibit strong Janzen-Connell spacing patterns as a result of actions of natural enemies.

### *ECOLOGICAL ANALOGUE SPECIES AND THE RESURRECTION OF EXTINCT INTERACTIONS*

One way of recreating some of the lost seed dispersal dynamics is to introduce extant species that are ecologically analogous to extinct ones, to perform the same or similar

ecological functions as the extinct species. To some ecologists and conservation biologists this idea may be anathema; in a best-case scenario it could be seen as little more than an attempt to create a small-scale version of Jurassic Park, and in one worst-case scenario it runs the risk of introducing species that may become invasive and have unintended negative effects on the ecosystem. Recently, the use of ecological analogue species to recreate the Pleistocene megafauna in South and North America, and in the Sibirian Tundra has been the subject of a heated debate (Galetti 2004; Martin 2005; Zimov 2005; Donlan *et al.* 2005, 2006; Rubenstein *et al.* 2006; Caro in press), partly due to the complexity of the involved ecosystems, and partly because of the vast areas needed to sustain populations of the suggested large-bodied animals. In contrast, due to their relatively small size and relative simplicity of their native ecosystems, oceanic islands may be ideal systems in which to empirically explore the use of ecological analogue species in a conservation management context (Jones 2002; Steadman & Martin 2003).

### *Our study*

Our model system was the oceanic island of Mauritius because it faces most of the problems that affect oceanic island ecosystems in general. Mauritius has lost the majority of its original vertebrate frugivorous and seed-dispersing fauna, and some studies have suggested that missing seed dispersers could be contributing to the continued decline in many of the endangered Mauritian plant species (Vaughan & Wiehe 1941; Maunder *et al.* 2002; Cheke & Hume in press). Very few studies have directly addressed the role of extant or extinct seed dispersal interactions in forest dynamics in present-day Mauritius (but see Nyhagen *et al.* 2005). We used the critically endangered endemic tree *Syzygium mamillatum* (Myrtaceae) as our model organism to study the effect of missing seed dispersers in the dynamics of present-day native forests in Mauritius.

We focused on seed germination, and the establishment and survival of seedlings of *S. mamillatum*. We addressed the specific questions: Are seed germination, and seedling growth and survival of *S. mamillatum* affected by distance to maternal trees? If so, can we use extant frugivorous animals as ecological analogue species to resurrect lost forest dynamics and ameliorate the negative effects?

## MATERIALS AND METHODS

### *STUDY SITE*

The study was conducted in the Black River Gorges National Park in Mauritius between March 2004 and February 2006. The study site is a 24 ha fenced and weeded Conservation Management Area (CMA) that was established in the lower montane evergreen wet forest of Brise Fér in 1996 (20°22.5'S, 57°26'E, 570-600 m elevation). Outside the CMA, the native forest is heavily degraded by invasive species, mainly *Psidium cattleianum*, and *Ligustrum robustum*. Brise Fér forest lies on a relatively narrow plateau, approximately 1500 x 500 m in size, with the CMA located in the central eastern part (Fig. 1).

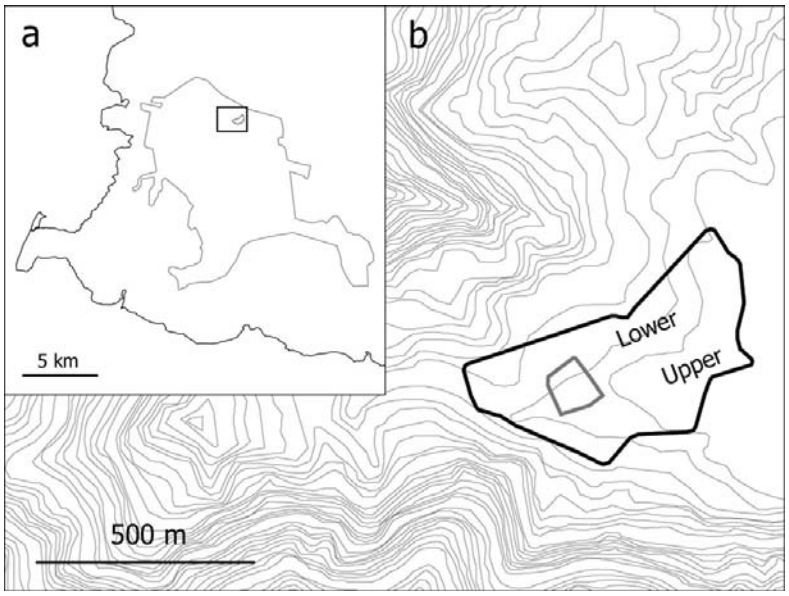



**FIGURE 1.** a) Map of the southwestern corner of Mauritius; borders of the National Park outlined in grey, and the enlarged part in b) indicated with a rectangle. b) Brise Fér plateau topography; each curve represents a change in altitude of 10 m. The fenced CMA is outlined in black, with the oldest part ('Old Plot') outlined in grey. The upper and lower plateaus of the eastern part of the CMA, where most of the *Syzygium mamillatum* trees are found, are labelled as 'Upper' and 'Lower', respectively.

Within the CMA the forest is not homogenous. It is roughly divided by a steep slope of 15–25 m in height into an upper southwestern plateau, characterised by a thin layer of top soil (erosion area), and a lower northern and northeastern plateau with deeper soil (accumulation area; Vaughan & Wiehe 1941). The forest structure reflects the soil difference, with a relatively low forest of 8–12 m on the upper plateau and a taller forest with much larger trees of 15–25 m on the lower plateau. The forest on the upper plateau is more open and dry than the forest on the lower plateau. Analysis of soil samples from the upper and lower plateau revealed significantly more clay on the lower plateau (16% vs.

11%), and more potassium on the lower plateau (55 mg/kg vs. 90 mg/kg), while all other soil properties were similar on the upper and lower plateau (unpubl. data).

*STUDY SPECIES*

*Syzygium mamillatum* (Myrtaceae) is a critically endangered endemic sub-canopy tree of 2.5–9 m in height, found in the upland Mauritian rainforests. Despite the striking basal cauliflory of *S. mamillatum* (most flowers on the lowest 1–1.5 m), the species was not described until 1987 (Bosser *et al.* 1987). While a few single trees or small stands are known from elsewhere in the national park (e.g. Macabé, Mare Longue and Mt. Cocotte, pers. obs.), the largest known population is located in Brise Fér. Previous surveys suggested a maximum of 20 *S. mamillatum* trees in this area, but during a focused search in July and August 2003 we found a total of 119 mature trees – representing the largest known population with 87% of all known mature trees of this species. The majority of trees in this population occur within the CMA (81 trees = 68%). Of the 81 adult trees in the CMA, 79 were upright and the remaining two trees had been partly knocked over by falling trees. Of the upright trees, 58 grew on the lower plateau and 21 on the upper plateau. The difference between upper and lower plateau in the CMA is apparent in the size of adult *S. mamillatum* trees. Trees growing on the lower plateau are larger than trees growing on the upper plateau, in terms of both height (all values are mean ± SD, compared with Student's t-tests; lower plateau = 6.2 ± 1.4 m, upper plateau = 5.2 ± 1.3 m, $t = 2.911$, $P = 0.006$) and diameter at breast height (lower plateau = 6.8 ± 2.2 cm, upper plateau = 5.3 ± 1.7 cm, $t = 3.08$, $P = 0.004$).

In another study (Kaiser *et al.* in press; Chapter 4), we investigated the pollination biology of *S. mamillatum*, and found it to be pollinated by endemic and introduced bird species. The average fruitset of trees in the CMA was 20–25%, with trees producing 1–520 ripe fruits (mean ± SD: 48 ± 100 fruits; N = 69 trees). Interestingly, on average, 73% of all ripe fruits on a tree developed on the lowest 30 cm of the trunk (Appendix Fig. 1A). After flower fertilisation in November–December, fruits take 4–5 months to ripen, and turn pale green or pale pink when ripe. They are 30–50 mm long, 15–30 mm wide at their widest point, and weigh some 4–10 g (Appendix Fig. 1B). Depending on ripeness, the pulp has the texture of a soft or very soft pear, with a relatively strong fermenting smell. In ripe fruits, the typically 2–4 seeds are easily separated from the pulp as a coherent whole, forming a rough 'ball' shape (Appendix Fig. 1C). Individual seeds are green without a hard seed coat, only a layer of semi-fibrous pulpy tissue (Appendix Fig. 1F). Every seed

has a well-defined line down the middle, separating the two cotyledons (Appendix Fig. 1D,F), along which they break quite easily if minimum force is applied. The cotyledons are bright green, and can stay that colour for up to 8–10 months under field conditions (pers. obs.).

We performed our study on *S. mamillatum* inside the CMA only. This was because the CMA contains the largest remaining population of *S. mamillatum*, and because almost no seedlings of native or endemic plant species survive to sapling stage in the heavily invaded forest outside of the CMAs, due to both competition with invasive plants and grazing/foraging by introduced animals (Lorence & Sussman 1986; Mauritian Wildlife Foundation unpublished database). It is most likely that survival of the native Mauritian forests will depend on weeding of invasive plant species and control of introduced animal species for the foreseeable future. Therefore, it is of greatest applied and immediate conservation importance to investigate and attempt to re-establish some of the lost dynamics in the remaining native Mauritian forests within the CMAs.

### *STATISTICAL ANALYSES*

Statistical models and methods used are specified in the relevant sections. All analyses were done with R.2.4.1 (R Development Core Team 2006).

### *NATURAL SEEDLING SURVEY*

During the search for adult trees in Brise Fér we only found seedlings around the base of adult trees, no more than 1 m away from the trunk. However, this may have been biased as we were not actively looking for seedlings on the forest floor while searching for adult trees. Therefore, we walked 10 transects on the upper plateau and 10 transects on the lower plateau. The parallel transects were 200–300 m long and oriented east-west, covering an area that included approximately 70–80% of the adult *S. mamillatum* in the CMA. Along each transect, we looked for *S. mamillatum* seedlings and saplings of any size within a 2 m belt transect. Furthermore, throughout the study period we opportunistically searched for seedlings and saplings throughout Brise Fér CMA.

### *FEEDING EXPERIMENTS WITH ECOLOGICAL ANALOGUE SPECIES*

Out of the multitude of frugivorous seed-dispersing ghosts in the Mauritian fauna (Cheke 1987; Cheke & Hume in press), we selected to resurrect and investigate the functional component of three of them, the dodo (*Raphus cucullatus*) and the two species of giant

tortoises, the high- or saddle-backed tortoise (*Cylindraspis triserrata*) and the domed tortoise (*C. inepta*). As dodo stand-ins, we used three domestic turkeys (*Meleagris gallopavo*). Turkeys have a powerful gizzard with grinding stones, like the dodo had (Hachisuka 1953), and no seeds from the 105 *S. mamillatum* fruits we fed to them passed through unharmed; we only found seed fragments of 1–2 mm in size. Therefore, we conclude that turkeys are not suitable analogue seed dispersers for *S. mamillatum*, and we present no further data from this part. As a stand-in for the two extinct giant tortoise species of Mauritius we used giant Aldabra tortoises, *Aldabrachelys gigantea*. All of the extinct Mascarene giant tortoise species have been reported to eat fruits and leaves (review in Cheke & Hume in press). Similarly, the Aldabra tortoise also feeds on all kinds of plant material, including fruits, and acts a seed disperser for several plant species in Aldabra (Hnatiuk 1978). While *Aldabrachelys* is probably not a direct sister genus of *Cylindraspis* (Austin & Arnold 2001), *A. gigantea* is likely to be the closest extant analogue of the extinct Mauritian species in ecological terms.

For the feeding experiment, we used three giant Aldabra tortoises from La Vanille Crocodile and Tortoise Park, Riviére des Anguilles (La Vanille hereafter), where they are usually part of a herd of some 200 adult tortoises in a large savannah-type enclosure. The three medium-sized tortoises, weighing approximately 70–100 kg each, were separated from the herd and kept in a smaller enclosure, where they were also being fed vegetables and other fruit throughout the feeding experiment. Forty fruits were fed to the three tortoises twice a week during four weeks, beginning on March 10, and finishing on April 5. A total of 320 ripe fruits from seven different *S. mamillatum* trees were fed to the tortoises (mean = 46 fruits/tree, range: 20–132 fruits/tree). *Syzygium mamillatum* fruits were fed whole to the tortoises (Appendix Fig. 1E). Opening the fruits and counting the seeds would potentially disrupt the layer of tissue keeping the seeds together (see Appendix Fig. 1C), which could in turn influence the effects of gut passage. Therefore, we estimated that the fruits fed to the tortoises contained a total of 685 seeds based on the average number of seeds per fruit (2.14 seeds, unpublished data). Tortoise faeces were collected daily in plastic bags at La Vanille from March 11 to May 5. Once a week, we collected the bags from La Vanille and examined the faeces. Whole *S. mamillatum* seeds and seed fragments, which were large enough to be identified as such (Appendix Fig. 1G). were extracted, counted and weighed.

*GERMINATION EXPERIMENTS*

We set up two different seed germination experiments in Brise Fér CMA. One in which we used whole fruits and manually depulped seeds, and another where we used tortoise gut-passed seeds from the feeding experiment.

For the first experiment, with whole fruits and manually depulped seeds, an unbalanced factorial design with four treatments was set up around 20 maternal *S. mamillatum* trees (if not stated otherwise, the replication for lower plateau is always N = 15 tree maternal trees and N = 5 maternal trees for upper plateau): (1) site of maternal trees (fixed factor PLATEAU with two levels: 'upper' and 'lower'), (2) distance from maternal tree (fixed factor DISTANCE with two levels: 'close' and 'away'), (3) propagule type (fixed factor PROPAGULE with two levels: 'seed' and 'fruit'), and (4) protection from vertebrate fruit- or seed predators (fixed factor CAGE with two levels: 'cage' and 'no cage'). The 20 maternal trees were used as a random factor in the analyses. This gave a total of 160 groups of seeds or fruits that will be referred to as 'patches'. Around each of the 20 maternal trees, the four close patches were set up 1 m away from the trunk in the four cardinal compas directions. The four away patches were set up in one of two different ways: either 20–25 m away from the maternal tree in the four cardinal directions, or 20–25 m away in a roughly perpendicular line with at least 6–8 m between patches. None of the away patches were set up closer than 25 m to any other *S. mamillatum* tree. We mainly used seeds and fruits from each of the 20 maternal trees. Only in a few cases there were not enough ripe fruits on the maternal tree, and here we supplemented with fruits or seeds from the nearest fruiting individual. In each of the seed patches we placed 4–7 seeds with the slimy seed coat attached, as this was difficult to remove from seeds without damaging them. The fruit patches consisted of three whole fruits. Both seeds and whole fruits in any one patch were placed directly on the ground in a 10 × 10 cm area. The cages were built with 0.5 × 0.5" wire mesh, 16 ×16 × 8 cm in size, and were fixed close to the ground by 6–8 metal cramps around the base (Appendix Fig. 1H). Cages were removed when the first seedling in a caged patch was about to touch the wire mesh, as we wanted to avoid any physical interference with seedling growth. This was done in October–December 2004, when almost all seedlings had emerged and seed predation was no longer considered important (see Appendix Fig. 1I for a typical patch of seedlings)

Seeds from the feeding experiments were also put out in Brise Fér CMA. Whole tortoise gut-passed seeds and several large fragments (half a seed, one cotyledon) were put out once a week in two caged plots ('plot' hereafter refers only to gut-passed seeds or

seedlings), one plot on the upper plateau and one on the lower plateau. Plots were placed a minimum of 25 m away from any *S. mamillatum* tree, and a minimum of 15 m away from each other. Each plot consisted of two 15 × 15 cm sections, one with whole seeds and one with seed fragments, spread out evenly. The two sections in each plot were roughly 30–40 cm apart and were covered with an amount of tortoise dung corresponding to the average tortoise turd size (roughly 6–8 × 10–15 cm), evenly spread out in a ca. 1 cm thick layer. Each plot was covered with a wire-mesh cage of roughly 100 × 100 × 20 cm in size. These cages were removed in December 2004. Two plots were set up each of the first four weeks and four plots were set up in the fifth week, where most seeds were collected. Thus, we had a total of 12 replicates, with N = 6 on the upper and N = 6 on the lower plateau.

*Initial seed numbers in patches*

As we put out whole fruits in the fruit patches we did not know how many seeds each fruit contained. Thus, we established a baseline number of seeds for each of these patches for use in subsequent analysis of germination patterns and germination success. This was done by scoring the number of whole seeds as soon as the pulp had decomposed, usually after 1-2 months. We investigated effects of PROPAGULE, DISTANCE, and CAGE on initial numbers of seeds per patch with an ANOVA.

*Germination patterns*

Seedling germination in patches and plots was recorded six times; roughly once per month for the first four months (where the majority of seeds germinated), and thereafter at different intervals, depending on timing of fieldwork in Mauritius. Germination was defined as the emergence of the first two leaves and not only the root growing into the soil, because many seeds never managed to get past the latter stage, but died before extending the shoot.

Due to the different number of maternal trees on the upper and lower plateau, the loss of several patches to feral pigs that broke into the CMA and to weeders working in the CMA, our experimental design was unbalanced. Furthermore, for the calculation and analyses of proportions of seeds germinated we needed to take the number of initial seeds in each patch into account. We therefore analysed seedling germination patterns with a generalised linear mixed-effects model with penalised quasi-likelihood (hereafter GLMM) (glmmPQL function in R.2.4.1, using the MASS library; Venables & Ripley 2002), with PLATEAU, DISTANCE, PROPAGULE, CAGE and Time as fixed effects, maternal tree as a

random effect, and using a binomial error structure. This method is robust for unbalanced data, and by using the 'cbind' command to calculate the germination proportions we weighted the sample sizes (number of seeds and seedlings per patch). Furthermore, we fitted an offset factor to take the different length of time intervals between germination censuses into account. Initially, we fitted a full model, whereupon non-significant higher-order interactions were removed and only the simplified model is presented (using the function 'anova.lme' from the nlme library to assess statistical significance).

*Overall germination success*

The overall germination success (proportion of initial seeds that germinated) was analysed by comparing the proportions of maximum number of seedlings out of the initial number of seeds in each patch with a GLMM, using the same fixed (except for Time) and random effects and error structure as above (for almost all patches the maximum number of seedlings was reached around December–January 2004). Initially, we fitted a full model, whereupon non-significant higher-order interactions were removed to simplify the model.

We compared germination success for gut-passed seeds to manually depulped seeds germinating in cages away from maternal trees only ('away seed cage' patches), using a GLM with a quasi-binomial error structure to account for over-dispersed data.

*SEEDLING MORPHOMETRICS*

We measured the height (from ground to where the uppermost leaf pair was attached to stem) and counted the leaves of all seedlings in each patch and plot twice; once in January 2005 and again in February 2006. For the analysis of the seedlings in patches, we used linear mixed-effects models with patch nested in maternal tree as random factors.

For seedling height in the plots we compared average seedling height per plot with height of seedlings in all patches (there was no significant difference in height between patches, see Results), averaged at the maternal tree level, with a Wilcoxon-Mann-Whitney test. Numbers of leaves per seedling in the plots was compared to numbers of leaves per seedling in away patches only, averaged at the maternal tree level, with a Wilcoxon-Mann-Whitney test. For both analyses, we pooled upper and lower plateau maternal trees and plots, as there were only few plots with seedlings germinating.

*SEEDLING DAMAGE*

We here define seedling damage broadly as a visible mark caused by anything that damages and/or feeds on the leaves. Levels of seedling damage were scored twice, in both patches and plots.

*First survey*

In the first survey in early January 2005, we randomly selected one seedling from each of the 160 patches where one or more seedlings had emerged and were still alive at this time (N = 117 patches). Due to the low number of emerged seedlings in the plots with gut-passed seeds, we here scored seedling damage on all seedlings and used plot averages in the analysis. We measured seedling height and counted the number of leaves for each seedling. We assessed the presence or absence of different categories of damage on each leaf, divided into seven categories: 1) leaf mines, 2) necrosis spots, 3) bite damage, 4) discolouration/wilting, 5) curled leaves, 6) fungus, and 7) scale insects. We analysed the proportion of total number of leaves affected by each of the damage categories, as well as the overall proportion of total number of leaves affected by one or more damage categories. We also investigated the diversity of damage categories suffered at the seedling level by analysing the proportion of all seven damage categories present at the seedling level. To weigh these proportions in relation to total number of leaves per seedlings, we used GLMMs with DISTANCE and PLATEAU as fixed effects and maternal tree as random effect, and with binomial error structures. A separate model was fitted for each of the seven damage categories, as well as one for overall proportion of damaged leaves and one for diversity of damage at the seedling level. We investigated possible interdependencies between damage categories with Pearson's correlation tests.

Seedling damage in the plots with seedlings from gut-passed seeds was compared to that of away seedling patches, using GLMMs, combining maternal tree and plot into one random effect. Here, we also fitted a separate model for each of the seven damage categories, one for overall proportion of damaged leaves, and one for diversity of damage at the seedling level.

*Second survey*

In the second seedling damage survey in mid-February 2005, we visually assessed the overall level of damage for all seedlings in each patch and each plot (n = 117 patches and 7 plots), using the following grouping: low (almost no damage, most to all seedlings

healthy, only few leaves damaged), medium (little damage, most seedlings healthy with few leaves damaged, one to a few seedlings damaged) and heavy (damage affecting most seedlings, leaves curled or wilting, one to more seedlings badly affected). For statistical analyses, damage levels were assigned a numerical value: low = 1, medium = 2, and heavy = 3. For patches, the results of the second survey were analysed with a linear mixed-effects model (Pinheiro & Bates 2000), using DISTANCE and PLATEAU as fixed effects and maternal tree as random effect. Seedling damage levels in the plots with seedlings from gut-passed seeds were compared to away patches, averaged at the maternal tree level, with a Student's t-test.

### *SEEDLING SURVIVAL*

Seedling survival was investigated by analysing the proportion of surviving seedlings in February 2006 in relation to the maximum number of seedlings in patches where at least one seedling had germinated (N = 132 patches). We used a GLMM with a binomial error structure. We initially fitted a full model with all factors (fixed: PLATEAU, DISTANCE, PROPAGULE, CAGE; random: maternal tree). Any significant or marginally significant factors or interactions between factors were retained, and included in a new minimum adequate model. Again, survival of seedlings in plots was compared to survival of away seedling patches only. We used a GLMM for the analysis, combining maternal tree and plot into one random effect.

## RESULTS

### *NATURAL SEEDLING SURVEY*

We found no natural *S. mamillatum* seedlings or saplings away from maternal trees on any of the transects. Natural seedlings were usually confined to a distance < 1 m away from the trunk of maternal trees; the only exception being for a few trees growing on slopes, where some seedlings were found up to 2–3 m downhill. We found the tallest natural seedlings (30–40 cm) around the three adult trees in the Old Plot (see Fig. 1), which has been weeded since 1987. However, these were all in a bad shape with only a handful of heavily damaged leaves left, and there were also several wilted and dead seedlings of the same size.

*FEEDING EXPERIMENTS WITH ECOLOGICAL ANALOGUE SPECIES*

Of the estimated total of 685 seeds fed to the giant tortoises, 108 (15.8%) passed unharmed, and we recovered an additional 419 fragments with a total weight of 143.9 g, corresponding to approximately 197 seeds (28.8%). Thus, an estimated 380 seeds (55.4%) were digested, at least partly. Minimum gut passage time was 12 days (from first feeding March 10 to first seed defecated March 22), with a theoretical maximum of 43 days (from first feeding to last seed defecated April 22). Because we fed the giant tortoises continuously over several weeks, to avoid overfeeding them an unusual food item, we cannot calculate a mean gut passage time. However, the temporal distribution patterns of gut-passed seeds and seed fragments in relation to the period of feeding suggests a mean gut passage time of 2–3 weeks (Fig. 2).

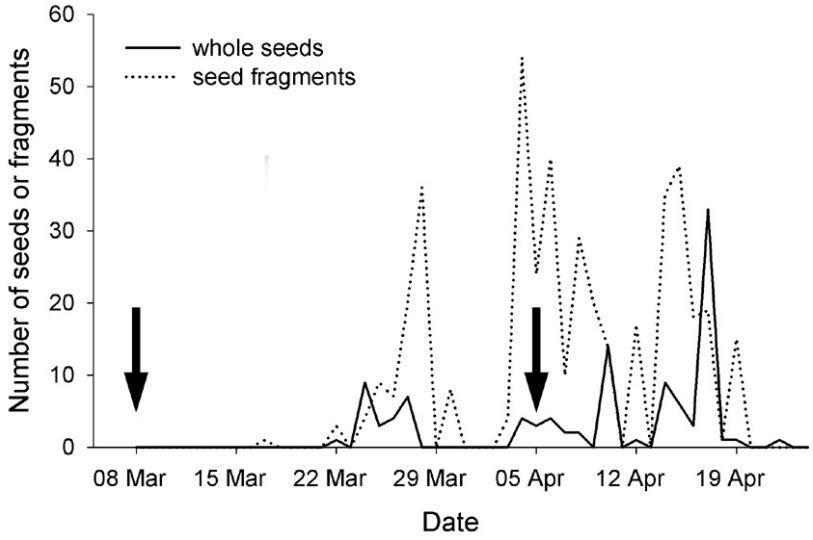



**FIGURE 2.** Gut-passage and excretion patterns of seeds and seed fragments from *Syzygium mamillatum* fruits fed to giant Aldabra tortoises. The two arrows indicate the beginning and the end of the feeding period, respectively.

*GERMINATION EXPERIMENTS*

*Initial seed numbers in patches*

Each patch contained 5-6 seeds when it was set up (mean ± SD = 5.4 ± 1.6 seeds). There was no significant effect of PROPAGULE ($F_{1, 147} = 0.142$, $P = 0.71$) or DISTANCE ($F_{1, 147} = 0.141$, $P = 0.71$) on initial numbers of seeds per patch. However, patches with cages held/contained on average more seeds than uncaged patches (5.7 ± 1.6 vs. 5.1 ± 1.5 seeds; $F_{1, 147} = 4.68$, $P = 0.03$). This difference, though, was only found for FRUIT (cage: 6.0 ± 2.1 seeds, no cage: 4.8 ± 2.0 seeds) and not for SEED (cage: 5.3 ± 0.89 seeds, no cage: 5.4

± 0.79 seeds; CAGE × PROPAGULE: $F_{1, 147} = 14.23$, $P = 0.02$). This suggests that pre-germination predation in patches was mostly restricted to whole fruits.

*Germination patterns*

There were no significant main effects of PLATEAU or DISTANCE on the overall germination pattern (Fig 3a,b; Table 1). However, seeds from whole fruits germinated both faster and with a higher proportion than manually depulped seeds (Fig. 3c; Table 1). Germination was faster with cage than without cage (Fig. 3d; Table 1), but only for seeds from whole fruits (Fig.3e; Table 1). Furthermore, there was a significant interaction between PROPAGULE and PLATEAU: while there was no difference in germination pattern for seeds from whole fruits on the upper and lower plateau, manually depulped seeds germinated worse on the upper than on the lower plateau (Fig. 3f; Table 1).

For gut-passed seeds, there were too few plots (N = 7 plots) where seeds germinated to perform germination pattern analyses with plot as a random factor. However, when plotting the cumulative germination for all gut-passed seeds pooled (Fig. 3c, N = 108 seeds), they appeared to germinate more slowly and at a lower proportion than both manually depulped seeds and seeds from whole fruits.

**TABLE 1.** Summary of the GLMM used to analyse *Syzygium mamillatum* seed germination patterns over time (see also Fig. 3) (DF = numerator degrees of freedom, denominator degrees of freedom).

| | DF | F | P |
|---|---|---|---|
| PLATEAU | 1, 18 | 0.72 | 0.408 |
| DISTANCE | 1, 130 | 0.24 | 0.627 |
| PROPAGULE | 1, 130 | 13.82 | **<0.001** |
| CAGE | 1, 130 | 1.90 | 0.171 |
| Time | 1, 772 | 335.98 | **<0.001** |
| PLATEAU × PROPAGULE | 1, 130 | 5.75 | **0.018** |
| PLATEAU × DISTANCE | 1, 130 | 0.01 | 0.920 |
| DISTANCE × PROPAGULE | 1, 130 | 0.03 | 0.853 |
| PLATEAU × CAGE | 1, 130 | 1.54 | 0.218 |
| DISTANCE × CAGE | 1, 130 | 0.12 | 0.732 |
| PROPAGULE × CAGE | 1, 130 | 5.24 | **0.024** |
| PLATEAU × Time | 1, 772 | 0.03 | 0.874 |
| DISTANCE × Time | 1, 772 | 0.62 | 0.432 |
| PROPAGULE × Time | 1, 772 | 10.89 | **0.001** |
| CAGE × Time | 1, 772 | 4.64 | **0.032** |
| PROPAGULE × CAGE × Time | 1, 772 | 3.94 | **0.048** |

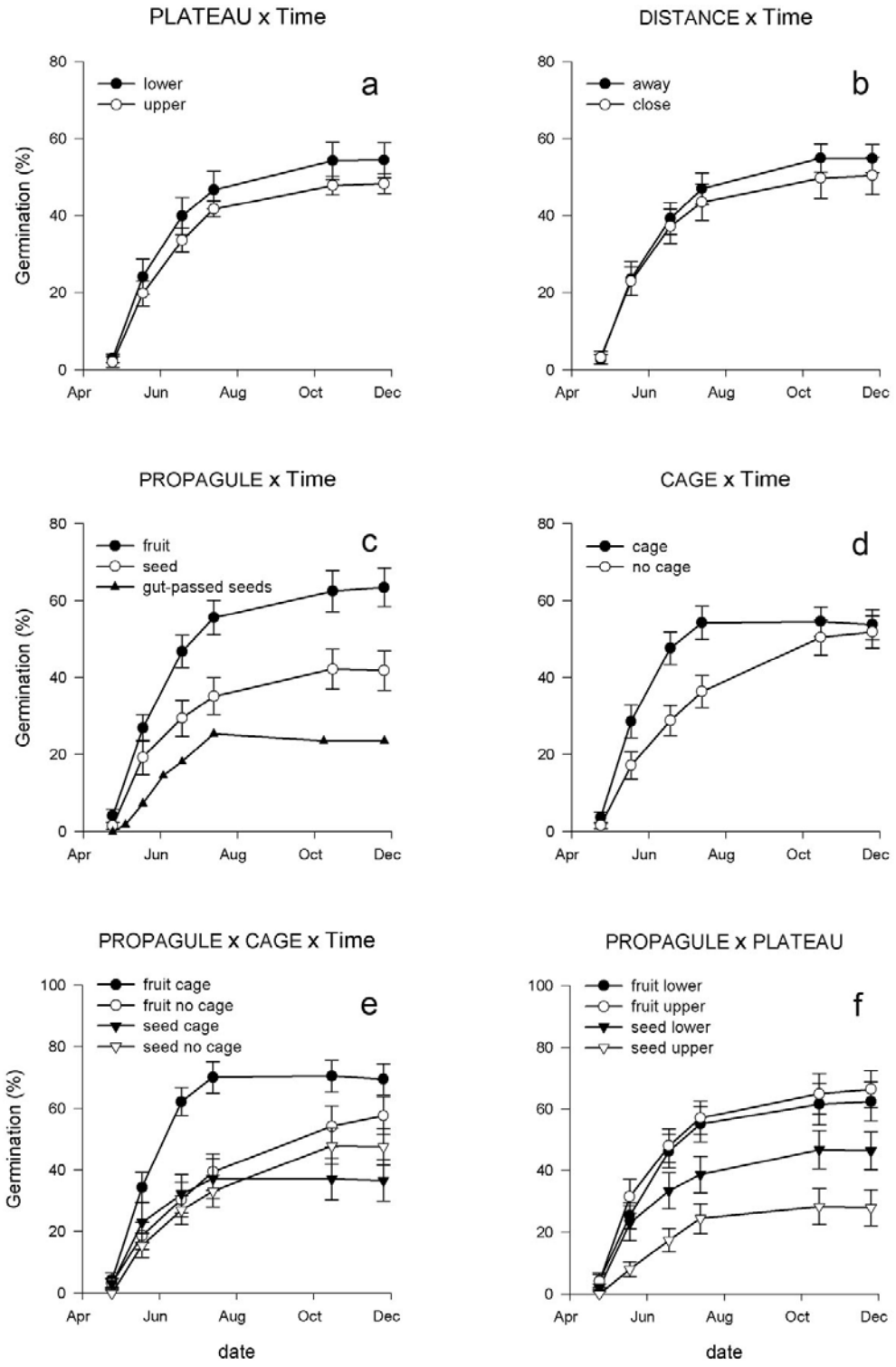



**FIGURE 3.** Seed germination patterns of *Syzygium mamillatum*. (a)-(d) illustrates the interactions with Time for each of the main effects (see Table 1). Values plotted are means ± 1 SE at the maternal tree level (N = 20 trees, except for PLATEAU which a sample size of 15 maternal trees on the lower plateau, and 5 maternal trees on the upper plateau). In (c), we have added the germination pattern of tortoise gut-passed seeds (overall proportion, seeds and seedlings pooled from all 12 gut-passed seed plots). (e) and (f) illustrate the two remaining significant interactions from Table 1.

*Overall germination success*

At maternal tree level a grand mean of 60.4 ± 0.03% (all means ± 1 SE) of the seeds germinated. In the GLMM, the only significant factor was PROPAGULE, with mean germination rates being 70.9 ± 0.04% for seeds from whole fruits and 49.3 ± 0.05% for manually depulped seeds ($F_{1, 131} = 20.86$, $P < 0.001$). There was a marginally significant interaction between PROPAGULE and PLATEAU ($F_{1, 131} = 2.96$, $P = 0.088$), explained by a difference in germination on upper versus lower plateau for manually depulped seeds (upper: 33.4 ± 0.1%, lower: 54.7 ± 0.1%) but not for seeds from whole fruits (upper: 71.4 ± 0.1%, lower: 70.7 ± 0.1%).

Seeds only germinated in seven of the 12 plots, and germination success of the gut-passed seeds in the plots was significantly lower than the 'away seed cage' patches used as the control (gut-passed seeds: 18.2 ± 7.0%, control: 47.4 ± 7.6%, $F_{1, 29} = 6.24$, $P = 0.018$). There appeared to be a negative effect of mean gut-passage time on germination success, with the first seeds collected germinating better than the last seeds (Fig. 4).

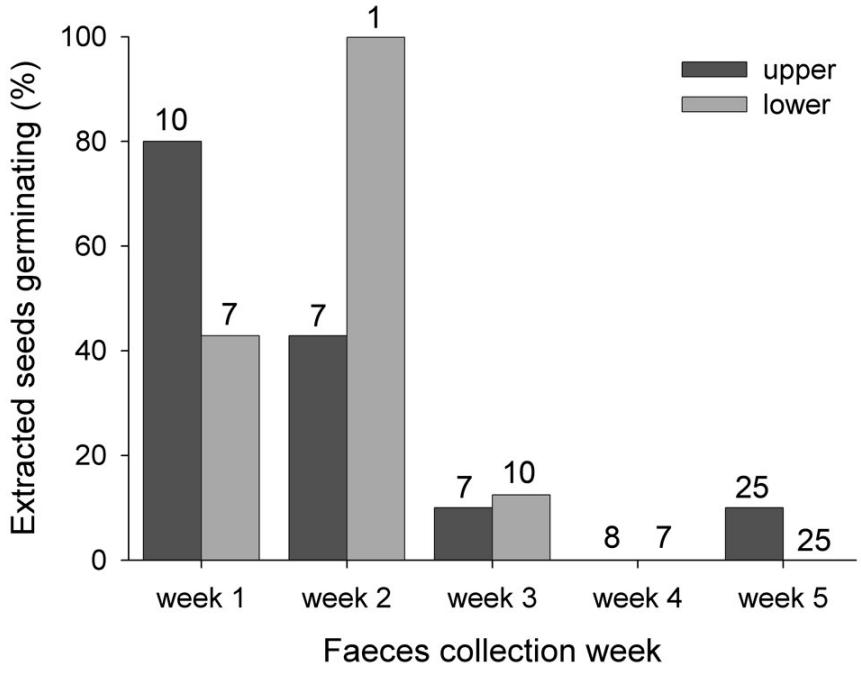



**FIGURE 4.** Percentage of tortoise gut-passed seeds germinating in relation to collection week. Numbers above the bars are the number of seeds sown in the forest on the upper and lower plateau, respectively.

*SEEDLING MORPHOMETRICS*

In 2005, DISTANCE had a highly significant effect on number of leaves per seedling, with more leaves per seedling away (7.6 ± 0.3 leaves) than close (6.2 ± 0.2; $F_{1, 97} = 12.54$, $P < 0.001$). There was no effect of DISTANCE on seedling height (overall mean height: 69.9 ± 1.4 mm; $F_{1, 97} = 0.78$, $P = 0.38$). The pattern was the same in 2006, with DISTANCE affecting number of leaves per seedling (away: 9.2 ± 0.5 leaves; close: 7.5 ± 0.4 leaves; $F_{1, 78} = 9.15$, $P = 0.003$), but not seedling height (overall mean height: 97.0 ± 2.6 mm; $F_{1, 78} = 0.11$, $P = 0.74$). Neither PLATEAU nor PLATEAU × DISTANCE interactions were statictically significant for height and number of leaves in 2005 or 2006 (all P-values > 0.10).

For seedlings from gut-passed seeds in the plots, we used maternal tree level averages of all patches as control group for height, and away patches as control group for

*SEEDLING DAMAGE*

*First survey*

In the first survey, when we scored the damage at the leaf level for one random seedling per patch, the effect of DISTANCE was significant for overall damage level, diversity of damage, and for most of the individual damage categories. A much higher proportion of leaves was damaged close to the maternal trees, compared with seedlings further away (Fig. 5; Table 2). Apart from a marginally significant interaction with DISTANCE for the damage category scale insects, PLATEAU was not a significant main effect and did not interact with DISTANCE for any other damage category.

The occurence of several damage categories were correlated (N = 117 seedlings; P-values given after sequentially Bonferroni corrections). Presence of white leaf fungus was significantly correlated with presence of both leaf mines ($r = 0.274$; $P = 0.048$), curled leaf ($r = 0.280$; $P < 0.036$) and necrosis spots ($r = 0.423$; $P < 0.001$). Presence of necrosis spots was significantly correlated with presence of scale insects ($r = 0.335$; $P < 0.001$), suggesting that the former may be caused by the latter. The least well-defined damage category, discolouration, was significantly correlated with the two damage categories affecting whole leaves, white fungus ($r = 0.331$; $P < 0.001$) and curled leaf ($r = 0.287$; $P = 0.034$), and is probably the final stage in overall damage before a leaf wilts and drops off.

Compared with seedlings away from maternal trees, seedlings from gut-passed seeds had a significantly lower total proportion of damaged leaves, whereas there was no significant difference in the diversity of damage categories (Fig. 5, Table 2). While there was a trend for seedlings from gut-passed seeds in comparison with seedlings away from

maternal trees to have a lower proportion of leaves damaged for almost all damage categories, the only significant difference was for necrosis spots (Fig. 5, Table 2).

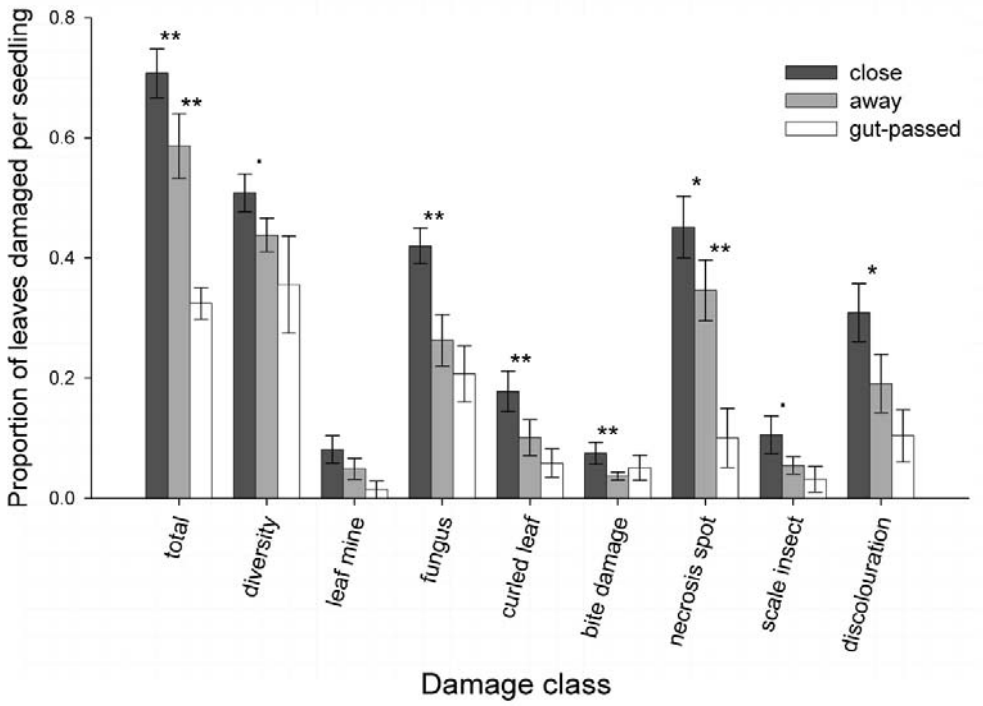



**FIGURE 5.** Seedling damage levels recorded in the first seedling damage survey in January 2005, expressed as proportions of total number of leaves per seedling suffering from the seven individual damage classes: 'leaf mine', 'fungus', 'curled leaf', 'bite damage', necrosis', 'scale insect', and 'discolouration'; 'total' means all seven damage classes pooled at the seedling level; 'diversity' means how many types of damage a seedling suffers from in proportion out of seven. Significant differences between close and away, and between away and gut-passed are marked above the corresponding pairs of bars ( . = 0.10 > P > 0.05; * = 0.05 > P > 0.005; ** = P < 0.005; see Table 2). All values for close and away seedlings are means ± 1 SE at the maternal tree level (N = 20 trees), values for seedlings from gut-passed seeds are means of the seven plots where seedlings emerged.

*Second survey*

As in the first survey, we found a strong effect of DISTANCE, with seedlings in patches close (N = 53) to the maternal trees scoring higher overall levels of damage than seedlings in patches away (N = 64) from the maternal trees (means ± 1 SE; close: 2.14 ± 0.10; away: 1.62 ± 0.12; linear mixed-effects model: $F_{1,95} = 22.3$, $P < 0.001$). PLATEAU had no significant effect on overall seedling damage level ($F_{1,18} = 1.92$, $P = 0.18$), nor was there a significant interaction between DISTANCE and PLATEAU ($F_{1,95} = 0.003$, $P = 0.95$).

**TABLE 2.** First seedling damage survey. The three first lines summarise the nine GLMMs used to analyse the first survey of damage for propagule seedlings, while the lowest line summarises the nine GLMMs used to compare damage levels of seedlings from gut-passed seeds in plots to seedlings in away patches (DF = numerator degrees of freedom, denominator degrees of freedom).

| | | total | | diversity | | leaf mine | | fungus | | curled leaf | | bite damage | | necrosis spot | | scale insect | | discolouration | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DF | F | P | F | P | F | P | F | P | F | P | F | P | F | P | F | P | F | P |
| PLATEAU | 1, 18 | 0.46 | 0.506 | 0.01 | 0.941 | 0.2 | 0.662 | 0.11 | 0.742 | 0.03 | 0.876 | 1.04 | 0.32 | 0.08 | 0.781 | 0.55 | 0.468 | 0.24 | 0.628 |
| DISTANCE | 1, 93 | 11.29 | **0.001** | 3.72 | *0.057* | 0.44 | 0.511 | 10.94 | **0.001** | 9.7 | **0.002** | 7.36 | **0.008** | 6.01 | **0.016** | 2.85 | *0.094* | 5.73 | **0.019** |
| PLATEAU × DISTANCE | 1, 93 | 0.79 | 0.377 | 0.67 | 0.415 | 1.6 | 0.208 | 1.62 | 0.207 | 0.12 | 0.725 | 2.57 | 0.113 | 0.88 | 0.35 | 2.96 | *0.089* | 0.14 | 0.713 |
| gut-passed vs. away | 1, 25 | 7.36 | **0.012** | 0.96 | 0.337 | 2.76 | 0.109 | 0.7 | 0.41 | 0.11 | 0.749 | 0.2 | 0.659 | 9.01 | **0.006** | 0.01 | 0.944 | 1.26 | 0.272 |

There was no difference in seedling damage between the seven plots with seedlings from tortoise gut-passed seeds and the away seedling patches (N = 64) used as control group (gut-passed seeds: 1.57 ± 0.20, ($F_{1,25}$ = 0.008, P = 0.93).

*SEEDLING SURVIVAL*

Seedling survival from when maximum number of seedlings had germinated in a patch (ca. Nov.–Dec. 2004) to February 2006 was strongly influenced by DISTANCE and marginally by PLATEAU (Table 3). DISTANCE had a highly significant overall effect on seedling survival, with much fewer seedlings surviving close to maternal trees compared to seedlings further away. Overall, PLATEAU had a marginal effect on seedling survival, with a slightly higher seedling survival on the lower plateau. However, there was a significant interaction between PLATEAU and DISTANCE, with seedling mortality being much higher close to maternal trees on the upper plateau than on the lower plateau.

Seedlings in the seven plots had the same survival rate as seedlings in the away patches used as a control group (plots: 77.4 ± 13.9 %; $F_{1,25}$ = 0.020, P = 0.89).

**TABLE 3.** Seedling survival from when maximum number of seedlings had germinated in a patch in November–December 2004 to February 2006, with corresponding test statistics from the GLMM analysis. Percentage survival is expressed as means ± 1 SE at level of the 20 maternal trees, with 15 trees on the lower and 5 trees on the upper plateau. (DF = numerator degrees of freedom, denominator degrees of freedom).

| Effects | Levels | Survival (%) | DF | F | P |
|---|---|---|---|---|---|
| PLATEAU | upper | 54.9 ± 7.1 | 1, 18 | 3.54 | 0.076 |
| | lower | 66.8 ± 4.0 | | | |
| DISTANCE | away | 78.1 ± 3.8 | 1, 110 | 29.49 | < 0.001 |
| | close | 48.0 ± 6.6 | | | |
| PLATEAU × DISTANCE | upper, close | 24.5 ± 11.9 | 1, 110 | 10.37 | 0.002 |
| | lower, close | 55.7 ± 6.2 | | | |
| | upper, away | 88.0 ± 3.7 | | | |
| | lower, away | 75.2 ± 4.6 | | | |

*Factors affecting survival*

Seedling damage recorded at the patch level (second damage survey) in February 2005 was a strong predictor for subsequent mortality from February 2005 to February 2006. Seedlings in patches with low damage level survived significantly better than those in

patches with medium and high damage levels (mean survival ± 1 SE; low: 90.1 ± 3.6%, N = 40 patches; medium: 79.2 ± 4.6%, N = 49 patches; high: 72.8 ± 8.9%,;N = 22 patches; GLMM with maternal tree as random factor: $F_{1,91} = 7.72$, P = 0.007).

There was no significant effect of numbers of seedlings in a patch on seedling survival in that patch from maximum number of seedlings in 2004 to February 2006 (GLMM with maternal tree as random factor, and using the maximum seedling number observed per patch, $F_{1,111} = 0.363$, P = 0.55).

## DISCUSSION

We found strong negative effects of proximity to maternal trees for seedling growth and survival in the critically endangered endemic Mauritian tree *Syzygium mamillatum*. This, to our knowledge, provides the first experimental evidence for a Janzen-Connell distance-dependent effect on the growth and survival of seedlings on an oceanic island. Our results clearly demonstrate the crucial importance of *S. mamillatum* propagules being dispersed away from the maternal trees. The fruitless search for natural seedlings and saplings showed that there are currently no frugivorous animals acting as efficient seed dispersers of *S. mamillatum*. We demonstrate that the Aldabra giant tortoise could be used as an ecological analogue species to provide a seed dispersal service, and thus resurrect the functional component of some of the extinct endemic frugivores in Mauritius.

### *SEED GERMINATION, AND SEEDLING GROWTH, DAMAGE AND SURVIVAL*

There was no difference in germination rate or germination speed between close and away patches. However, germination rate and speed, and overall germination success were strongly affected by propagule type. For several Mauritian fleshy-fruited tree species, it has been shown that removal of the fruit pulp is important for successful seed germination; if left on ripe fruits, the pulp often gets infected by fungi that spread into the seeds and destroy them (Wyse-Jackson *et al.* 1988; Nyhagen *et al.* 2005). Seed destruction or reduced seed germination rate as a result of fungal infestations of the pulp is a common pattern found elsewhere as well (e.g. Oliveira *et al.* 1995). However, this does not seem to be the case for *S. mamillatum*. On the contrary, our results show that seeds from whole fruits germinate faster and at a higher rate than manually depulped seeds. This could be due to a high level of essential oils with anti-fungal properties found in many plants from the family of Myrtaceae (e.g. in fruits of *S. cordatum*; Pretorius *et al.* 2002). Moreover, some *Syzygium* species in Australia germinate better if fruits have been fermenting

(Beardsell *et al.* 1993), and a similar effect is possible for *S. mamillatum*. Lastly, because *S. mamillatum* seeds have no hard endocarp, the pulp may protect seeds from rapid desiccation that could force them into dormancy. The latter is likely to be the main explanation in our case, as manually depulped seeds germinated more slowly and at a lower rate on the upper plateau, which is a drier and warmer habitat than the lower plateau. Seeds from whole fruits, on the other hand, germinated equally well on the upper and lower plateau.

The effects of caging on germination were more complex. Initially, the cages did protect the propagules against predation by larger animals. However, this effect was only seen for whole fruits, where initial counts of seeds (after the pulp had rotted away) were lower for non-caged than for caged patches. This is probably due to introduced ground-foraging animals, such as rats or tenrecs, grabbing whole fruits in each non-caged fruit patch. Caging also had a strong effect on germination speed, but only for seeds from whole fruits. This is puzzling, but may be due to foraging animals selectively disturbing decomposing fruit (where seeds often already had started germinating, pers. obs.), and not single seeds.

Most importantly, contrary to seedling germination patterns, seedling damage levels and subsequent seedling survival were strongly affected by proximity to maternal trees. Seedlings suffered less damage and had higher survival rates when growing away from maternal trees. Furthermore, seedlings had more leaves away from maternal trees. The overall poorer growth conditions on the upper plateau, less soil of a worse quality and a drier habitat, were also evident in seedling growth and seedling survival.

### *THE EFFECTS OF TORTOISE GUT-PASSAGE*

Despite the relatively low number of *S. mamillatum* seeds passing undamaged through the tortoises, there are at least two mitigating factors that could contribute to a high overall seedling success for gut-passed seeds in the medium to long term: Firstly, seeds are almost certainly dispersed away from areas with high seedling mortality near adult trees. Secondly, gut-passed seeds are deposited in a favourable microclimate with plenty of nutrients, which our results show leads to better growth and a lower susceptibility to natural enemies. Seedlings from gut-passed seeds grew taller, had more leaves, and suffered less leaf damage than control seedlings in both damage surveys. This could be because the higher nutrient status means that more secondary compounds to deter natural enemies can be produced (Coley *et al.* 1985).

Our estimated mean gut-passage time of 2–3 weeks is comparable to results from other studies of giant tortoises (Rick & Bowman 1961; Hamilton & Coe 1982). As illustrated in Fig. 4, longer gut-passage times appears to decrease germination success. All else being equal, the seeds extracted from tortoise faeces in week five were likely to have spent longer in tortoise guts than seeds extracted in week one. A similar trend towards lower germination success with increasing tortoise gut-passage time was noted for *Lycopersicon cheesmanii* seeds ingested by Galápagos tortoises (Rick & Bowman 1961). Galápagos tortoises sometimes ingest sand, gravel or pieces of wood, and these may cause a mechanical break-up of food items that could aid digestion (Rick & Bowman 1961). We did find both sand and pebbles in the faeces of the Aldabra tortoises, and it is likely that this was partly responsible for the break-up of many of the *S. mamillatum* seeds.

Compared to seeds of many other Mauritian fleshy-fruited plant species, *S. mamillatum* is probably one of the 'worst' species we could have chosen as our model organism. Its seeds have no hard endocarp to protect the cotyledons and embryo, and they therefore break apart easily, destrying the seed. In contrast, the seeds of most other Mauritian fleshy-fruited plant species have some sort of harder seed coat that would provide ample protection during tortoise gut-passage. For example, on the small off-shore islet Ile aux Aigrettes, where Aldabra tortoises are used in a grazing study, they also eat the fallen fruits of the endangered *Diospyros egrettarum* (Ebenaceae). The seeds of this species have a thin but hard and smooth seed coat and pass through the tortoises unscathed (Appendix Fig. 2), germinating very well afterwards (pers. obs.). It is therefore very likely that giant Aldabra tortoises will be able to perform well as seed dispersers of many Mauritian plant species.

Lastly, in any study that aims to investigate seed germination and seedling establishment and the influence of gut-passage on endangered species in conservation areas, it is important to do so in the field, rather than in nurseries or greenhouses, where conditions can be very different from those in the field. Rodriguez-Perez *et al.* (2005) found that germination rates of a species after gut-passage through birds and lizards could vary greatly between field- and garden sites, sometimes with completely opposite patterns. Furthermore, it is important to include all possible control groups to seeds from gut-passage experiments; that is, not only manually depulped seeds but also whole fruits or infructescences, a setup that is regrettably still not the norm in most experimental seed dispersal studies (Samuels & Levey 2005). Failure to use a proper protocol may lead to wrong recommendations for future conservation management strategies.

*JANZEN-CONNELL PATTERNS ON MAURITIUS AND OTHER OCEANIC ISLANDS*

Our results clearly demonstrate that the predictions of the Janzen-Connell model apply to seedling survival of *S. mamillatum* in Mauritius. However, more studies on other plant species in Mauritius and, above all, more studies on other oceanic islands are needed before any generalisations can be made. With our study, we were able to identify some of the potential drivers of Janzen-Connell patterns in *S. mamillatum* seedling damage and subsequent mortality. Parts of the damage were clearly related to activity by insects (mines, scale insects and probably most of the small necrosis spots) and fungi (white fungus and maybe curled leaves). Interdependence of damage categories is very likely, and we found significant correlations between occurrences of several seedling damage categories (Table 3). For example, the presence of leaf fungus was significantly correlated with presence of two of the mechanical damage categories, leaf mines and necrosis spots. This is in line with García-Guzman & Dirzo (2001), who showed that fungal pathogens in a tropical rainforest required insect damage to infect plants.

Of course, we cannot be sure that seedling damage and mortality is primarily related to natural enemies, rather than, for example, seedling competition. However, the patterns of increased damage were evident even in the patches with one solitary seedling or few seedlings that did not grow in a tight clump. Here, seedling densities are likely to be below levels that could lead to seedling competition (Clark & Clark 1984). In fact, it has been suggested that competition for resources between seedlings is unlikely to be a major contributor to seedling mortality in tropical forests, at least for young seedlings (Wright 2002). Therefore, it is most likely that the differences in *S. mamillatum* seedling damage and mortality in relation to distance from maternal trees are a result of corresponding differences in activity levels of natural enemies near to and away from maternal trees.

*Specialist or generalist natural enemies as drivers of Janzen-Connell patterns in Mauritius and other oceanic islands?*

We were not able to identify the natural enemies that caused the seedling damage. This is an obvious limitation of our study, and more investigations on the identity and specificity of natural enemies of plants on islands are much needed (Ribeiro *et al.* 2005). Therefore, we cannot speculate on the overall relative importance of generalists and specialists in our study system. However, if host-specificity of natural enemies in Mauritius is apparent at

the family or genus level, which is often the case for insect herbivores in tropical forests (Novotny & Basset 2005), then *S. mamillatum* and other endangered Myrtaceae species in Mauritius may be especially vulnerable. Two of the main invasive plant species in Mauritius are from the same genus and family as *S. mamillatum*, respectively (*S. jambos* and *Psidium cattleianum*). These abundant invasives could act as reservoirs of natural enemies in the invaded parts of the forest, which could lead to a high migration rate of natural enemies into the CMAs.

In general, islands are said to harbour simple ecosystems – in which case we could expect more generalist than specialist natural enemies. However, even generalist natural enemies can also be density- or even distance-responsive (Janzen 1970, Clark & Clark 1984). Moreover, with increasing age, islands harbour more species-rich and complex plant communities. In turn, this creates more niches for specialised herbivores. The incidence of specialist herbivores on an oceanic island is therefore likely to depend on the age of the island (e.g. Borges & Brown 1999; Gillespie & Roderick 2002). As a result, we may expect to find specialist-driven Janzen-Connell patterns more often on old than on young oceanic islands. More studies on the prevalence of generalist and specialist natural enemies on oceanic islands, and how they affect regeneration of plant species, are clearly needed.

### *THE USE OF ECOLOGICAL ANALOGUE SPECIES TO RESURRECT LOST SEED DISPERSAL INTERACTIONS ON OCEANIC ISLANDS*

In our study we assessed the use of Aldadra tortoises by using captive animals for feeding experiments, and subsequently putting seeds and faeces out into the CMA. This is a good approach for intial assessments of the suitability and functioning of ecological analogue seed dispersers. However, it contributes only little to restoring natural dynamics in the forest; ultimately, we need to release candidate ecological analogue species into the habitat in which we want to resurrect the lost interactions.

On Curieuse Island in the Seychelles, translocated Aldabra tortoises readily ate fruits of plants they had not encountered before (Hambler 1994). However, they dispersed seeds of invasive species, too, but this would not pose a problem within the weeded CMAs in Mauritius. One major advantage of using giant tortoises as ecological analogues is that it is relatively easy to monitor them and, if necessary, to add or remove tortoises, thus adjusting their impact on the habitat (Jones 2002).

There are several important points to consider when selecting candidate species for release as ecological analogues within conservation management areas on oceanic islands. Firstly, although it may be tempting to look for the closest living relative of the extinct species, an evolutionarily close extant species species is not necessarily a good ecological analogue (Jones 2002). That is, close taxonomical affinity does not automatically translate into ecological similarity. This is in particular the case on oceanic islands, which are famous for the large number of adaptive radiations. Secondly, it would not make sense to release ecological analogue species without having addressed the factors that resulted in the extinction of the original species in the first place. The latter point is already the main focus of many CMAs on oceanic islands; introduced predators and invasive competitors have been eradicated or are being controlled or excluded, especially on smaller offshore islets and fenced habitats on main islands (e.g. Towns *et al.* 1990; Nogales *et al.* 2004).

It is ironic that one of the first and best known but poorly executed studies of a plant and its extinct seed disperser – and the use of an ecological analogue species to replace it – is from Mauritius. The famous Dodo and Tambalacoque story (Temple 1977) has been cited frequently in the ecological literature as an example of a disrupted mutualism, but suffers from serious flaws (Witmer & Cheke 1991; Cheke & Hume in press), and fails by a large margin to demonstrate anything like the 'obligatory mutualism' it suggests. There is more than one candidate ghost in the Mauritian frugivore fauna that could have dispersed the Tambalacoque seeds; giant tortoises or giant skinks, for example (Iverson 1987; Witmer & Cheke 1991). There are even extant fruitbats that are capable of dispersing the large fruits (V. Florens, pers. comm.). This story does serve to prove a very important point, though: Most seed dispersal mutualisms are not specialised. Only rarely does one plant species depend on one animal species for dispersal, and only rarely does one frugivore depend on one plant species for food (Howe & Smallwood 1982). Hence, one ecological analogue seed-dispersing species is likely to benefit more than one plant species.

## CONCLUSIONS

Many studies have pointed out the important roles of either disrupted seed dispersal mutualisms (e.g. Bond 1994; Traveset & Riera 2005) or natural enemies (Gilbert & Hubbell 1996; Bevill *et al.* 1999) in the conservation of rare plants. With our study we highlight the combined potentially greater importance of both for endangered plants on oceanic islands compared to mainland habitats. Conservation management of endangered

plants on oceanic islands should take both missing seed dispersers and resulting Janzen-Connell patterns in seedling growth and mortality into account. We suggest that one way of mitigating a lack of dispersal and improving seedling performance is to use ecological analogue frugivorous species *in situ*. Furthermore, it is important for future studies to expand on the importance of Janzen-Connell patterns in conservation management areas in Mauritius and other oceanic islands to include other, more numerous species, to be able to investigate density- as well as distance-dependent effects (Wills *et al.* 1997).

Lastly, our suggestions for using ecological analogue species in the conservation management of endangered oceanic island species may be expanded to mainland habitat fragments, which often suffer from locally extinct seed dispersal interactions (e.g. Cordeiro & Howe 2001; Galetti *et al.* 2006).

## ACKNOWLEDGEMENTS

We want to express our gratitude to the National Parks and Conservation Service of Mauritius for allowing us to live and work in the National Park, to Owen Griffiths, Gilbert Moutia, and the rest of the staff at la Vanille Crocodile and Tortoise Park, for allowing us to use some of their precious tortoises, and to the volunteers and staff at the Mauritian Wildlife Foundation for never-failing support and friendship. Furthermore, we thank Jannie Linnebjerg, Lotte Reiter, Heine Kiesbüy, Kelly Edmunds and Nancy Bunbury for help in the field, and Christopher Philipson, Simone Härri, Andy Hector, and Robert Bagchi for statistical advice. The manuscript benefited much from critical readings and comments by Nancy Bunbury, Mauro Galetti, and Anna Traveset. The project was funded by the Swiss National Science Foundation (grant no. 631-065950 to CBM).

## REFERENCES

AREVALO, J.R. & FERNANDEZ-PALACIOS, J.M. (2003) Spatial patterns of trees and juveniles in a laurel forest of Tenerife, Canary Islands. *Plant Ecology* 165: 1-10.

AUSTIN, J.J. & ARNOLD, E.N. (2001) Ancient mitochondrial DNA and morphology elucidate an extinct island radiation of Indian Ocean giant tortoises (Cylindraspis). *Proceedings of the Royal Society of London Series B-Biological Sciences* 268: 2515-2523.

BEARDSELL, D.V., O´BRIEN, S.P., WILLIAMS, E.G., KNOX, R.B. & CALDER, D.M. (1993) Reproductive biology of Australian Myrtaceae. *Australian Journal of Botany* 41: 511-526.

BEVILL, R.L., LOUDA, S.M. & STANFORTH, L.M. (1999) Protection from natural enemies in managing rare plant species. *Conservation Biology* 13: 1323-1331.

BOND, W.J. (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society London – Series B Biological Sciences* 344: 83-90.

BORGES, P.A.V. & BROWN, V.K. (1999) Effect of island geological age on the arthropod species richness of Azorean pastures. *Biological Journal of the Linnean Society* 66: 373-410.

BOSSER, J., CADET, T. & GUÉHO, J. (1987) Nouvelles observations sur des *Syzygium* (Myrtaceae) des Mascareignes. *Adansonia* 9: 29-38.

CARO, T. (in press) The Pleistocene re-wilding gambit. *Trends in Ecology & Evolution*.

CHEKE, A.S. (1987) An ecological history of the Mascarene Islands, with particular reference to extinctions and introductions of land vertebrates. In: *Studies of Mascarene Island birds* (Diamond A.W., ed). Cambridge: Cambridge University Press; 5-89.

CHEKE, A.S. & HUME, J.P. (in press) *Lost land of the Dodo*. London, UK: Christopher Helm.

CLARK, D.A. & CLARK, D.B. (1981) Effects of seed dispersal by animals on the regeneration of *Bursera gravevolens* (Burseraceae) on Santa Fe Island, Galápagos. *Oecologia* 49: 73-75.

CLARK, D.A. & CLARK, D.B. (1984) Spacing dynamics of a tropical rain forest tree: Evaluation of the Janzen-Connell model. *American Naturalist* 124: 769-788.

COLEY, P.D., BRYANT, J.P. & CHAPIN, F.S. (1985) Resource availability and plant antiherbivore defense. *Science* 230: 895-899.

CONNELL, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: *Dynamics of populations* (Boer P.J.D., Gradwell G.R., eds). Wageningen, Netherlands: Centre for Agricultural Publication and Documentation; 298-312.

CORDEIRO, N.J. & HOWE, H.F. (2001) Low recruitment of trees dispersed by animals in African forest fragments. *Conservation Biology* 15: 1733-1741.

COX, P.A., ELMQVIST, T., PIERSON, E.D. & RAINEY, W.E. (1991) Flying foxes as strong interactors in South Pacific island ecosystems: a conservation hypothesis. *Conservation Biology* 5: 448-454.

DONLAN, J. (2005) Re-wilding North America. *Nature* 436: 913-914.

GALETTI, M. (2004) Parks of the Pleistocene: recreating the Cerrado and the Pantanal with megafauna. *Natureza & Conservação* 2: 93-100.

GALETTI, M., DONATTI, C.I., PIRES, A.S., GUIMARAES, P.R. & JORDANO, P. (2006) Seed survival and dispersal of an endemic Atlantic forest palm: the combined effects of defaunation and forest fragmentation. *Botanical Journal of the Linnean Society* 151: 141-149.

GARCÍA-GUZMAN, G. & DIRZO, R. (2001) Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: incidence, spatiotemporal variation, and mechanisms of infection. *American Journal of Botany* 88: 634-645.

GILBERT, G.S. & HUBBELL, S.P. (1996) Plant diseases and the conservation of tropical forests. *Bioscience* 46: 98-106.

GILLESPIE, R.G. & RODERICK, G.K. (2002) Arthropods on islands: Colonization, speciation, and conservation. *Annual Review of Entomology* 47: 595-632.

GRANT, P.R. (1998) *Evolution on islands*. Oxford, UK.

HACHISUKA, M. (1953) *The Dodo and kindred birds, or the extinct birds of the Mascarene Islands*. London, UK: H.F. & G.Witherby.

HAMBLER, C. (1994) Giant tortoise *Geochelone gigantea* translocation to Curieuse Island (Seychelles): success or failure? *Biological Conservation* 69: 293-299.

HAMILTON, J. & COE, M. (1982) Feeding, digestion and assimilation of a population of giant tortoises (*Geochelone gigantea* (Schweigger)) on Aldabra atoll. *Journal of Arid Environments* 5: 127-144.

HARMS, K.E., WRIGHT, S.J., CALDERON, O., HERNANDEZ, A. & HERRE, E.A. (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature* 404: 493-495.

HNATIUK, S.H. (1978) Plant dispersal by the Aldabran giant tortoise, *Geochelone gigantea* (Schweigger). *Oecologia* 36: 345-350.

HOWE, H.F. & MIRITI, M.N. (2000) No question: seed dispersal matters. *Trends in Ecology & Evolution* 15: 434-436.

HOWE, H.F. & SMALLWOOD, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201-228.

IVERSON, J.B. (1987) Tortoises, Not Dodos, and the Tambalacoque Tree. *Journal of Herpetology* 21: 229-230.

JANZEN, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist* 104: 501-529.

JANZEN, D.H. (1971) Seed predation by animals. *Annual Review of Ecology and Systematics* 2: 465-492.

JONES, C.G. (2002) Reptiles and Amphibians. In: *Handbook of Ecological Restoration* (Perrow M.R., Davy A.J., eds). Cambridge: Cambridge University Press; 355-375.

KAISER, C. N., HANSEN, D. M. & MÜLLER, C. B. (in press). Habitat structure affects reproductive success of the rare endemic tree *Syzygium mamillatum* (Myrtaceae) in restored and unrestored sites in Mauritius. *Biotropica*.

LAWTON, J. (1995) Ecology of the afterlife. *Oikos* 73: 145-147.

LEE, M.A.B. (1985) The dispersal of *Pandanus tectorius* by the land crab *Cardisoma carnifex*. *Oikos* 45: 169-173.

LORENCE, D.H. & SUSSMAN, R.W. (1986) Exotic species invasion into Mauritius wet forest remnants. *Journal of Tropical Ecology* 2: 147-162.

MARTIN, P.S. (2005) *Twilight of the mammoths - Ice age extinctions and the rewilding of America*. California University Press.

MAUNDER, M., PAGE, W., MAUREMOOTOO, J., PAYENDEE, R., MUNGROO, Y., MALJKOVIC, A., VERICEL, C. & LYTE, B. (2002) The decline and conservation management of the threatened endemic palms of the Mascarene Islands. *Oryx* 36: 56-65.

NOGALES, M., MARTIN, A., TERSHY, B.R., DONLAN, C.J., WITCH, D., PUERTA, N., WOOD, B. & ALONSO, J. (2004) A review of feral cat eradication on islands. *Conservation Biology* 18: 310-319.

NOVOTNY, V. & BASSET, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B-Biological Sciences* 272: 1083-1090.

NYHAGEN, D.F., TURNBULL, S.D., OLESEN, J.M. & JONES, C.G. (2005) An investigation into the role of the Mauritian flying fox, *Pteropus niger*, in forest regeneration. *Biological Conservation* 122: 491-497.

OLIVEIRA, P.S., GALETTI, M., PEDRONI, F. & MORELLATO, L.P.C. (1995) Seed cleaning by *Mycocepurus goeldii* ants (Attini) facilitates germination in *Hymenaea courbaril* (Caesalpiniaceae). *Biotropica* 27: 518-522.

PRETORIUS, J.C., ZIETSMAN, P.C. & EKSTEEN, D. (2002) Fungitoxic properties of selected South African plant species against plant pathogens of economic importance in agriculture. *Annals of Applied Biology* 141: 117-124.

R DEVELOPMENT CORE TEAM (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

REID, N. (1989) Dispersal of mistletoes by honeyeaters and flowerpeckers: components of seed dispersal quality. *Ecology* 70: 137-145.

RIBEIRO, S.P., BORGES, P.A.V., GASPAR, C., MELO, C., SERRANO, A.R.M., AMARAL, J., AGUIAR, C., ANDRE, G. & QUARTAU, J.A. (2005) Canopy insect herbivores in the Azorean Laurisilva forests: key host plant species in a highly generalist insect community. *Ecography* 28: 315-330.

RICK, C.M. & BOWMAN, R.I. (1961) Galápagos tomatoes and tortoises. *Evolution* 15: 407-417.

RODRÍGUEZ-PEREZ, J., RIERA, N. & TRAVESET, A. (2005) Effect of seed passage through birds and lizards on emergence rate of Mediterranean species: differences between natural and controlled conditions. *Functional Ecology* 19: 699-706.

RUBENSTEIN, D. R., D. I. RUBENSTEIN, P. W. SHERMAN, AND T. A. GAVIN. 2006. Pleistocene park: Does re-wilding North America represent sound conservation for the 21st century? Biological Conservation **132**:232-238.

SAMUELS, I.A. & LEVEY, D.J. (2005) Effects of gut passage on seed germination: do experiments answer the questions they ask? *Functional Ecology* 19: 365-368.

STEADMAN, D.W. & MARTIN, P.S. (2003) The late Quaternary extinction and future resurrection of birds on Pacific islands. *Earth-Science Reviews* 61: 133-147.

TEMPLE, S.A. (1977) Plant-animal mutualism: Coevolution with Dodo leads to near extinction of plant. *Science* 97: 885-886.

TOWNS, D.R., DAUGHERTY, C.H. & ATKINSON, I.A.E. (1990) *Ecological restoration of New Zealand islands*. Wellington, New Zealand: Department of Conservation.

TRAVESET, A. & RIERA, N. (2005) Disruption of a plant-lizard seed dispersal system and Its ecological effects on a threatened endemic plant in the Balearic Islands. *Conservation Biology* 19: 421-431.

VAUGHAN, R.E. & WIEHE, P.O. (1941) Studies on the vegetation of Mauritius: III. The structure and development of the upland climax forest. *Journal of Ecology* 29: 127-160.

VENABLES, W.N. & RIPLEY, B.D. (2002) *Modern applied statistics with S.*, Fourth Edition ed. New York: Springer.

VITOUSEK, P.M., LOOPE, L.L. & ADSERSEN, H. (1995) *Islands: Biological diversity and ecosystem function*. Heidelberg, Germany: Springer Verlag.

WILES, G.J., SCHREINER, I.H., NAFUS, D., JURGENSEN, L.K. & MANGLONA, J.C. (1996) The status, biology, and conservation of Serianthes nelsonii (Fabaceae), an endangered Micronesian tree. *Biological Conservation* 76: 229-239.

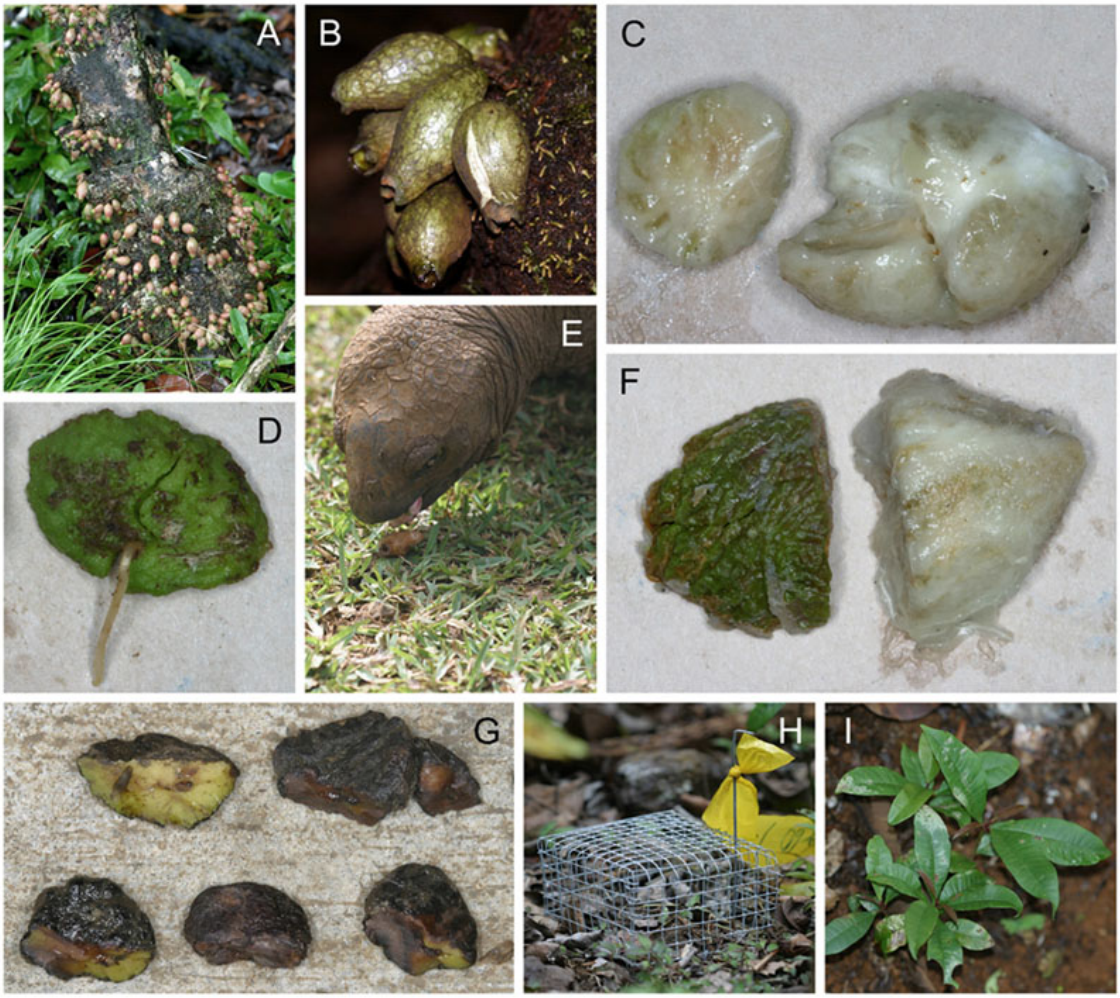
WILLS, C., CONDIT, R., FOSTER, R.B. & HUBBELL, S.P. (1997) Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. *PNAS* 94: 1252-1257.

WITMER, M.C. & CHEKE, A.S. (1991) The dodo and the tambalacoque tree: an obligate mutualism reconsidered. *Oikos* 61: 133-137.

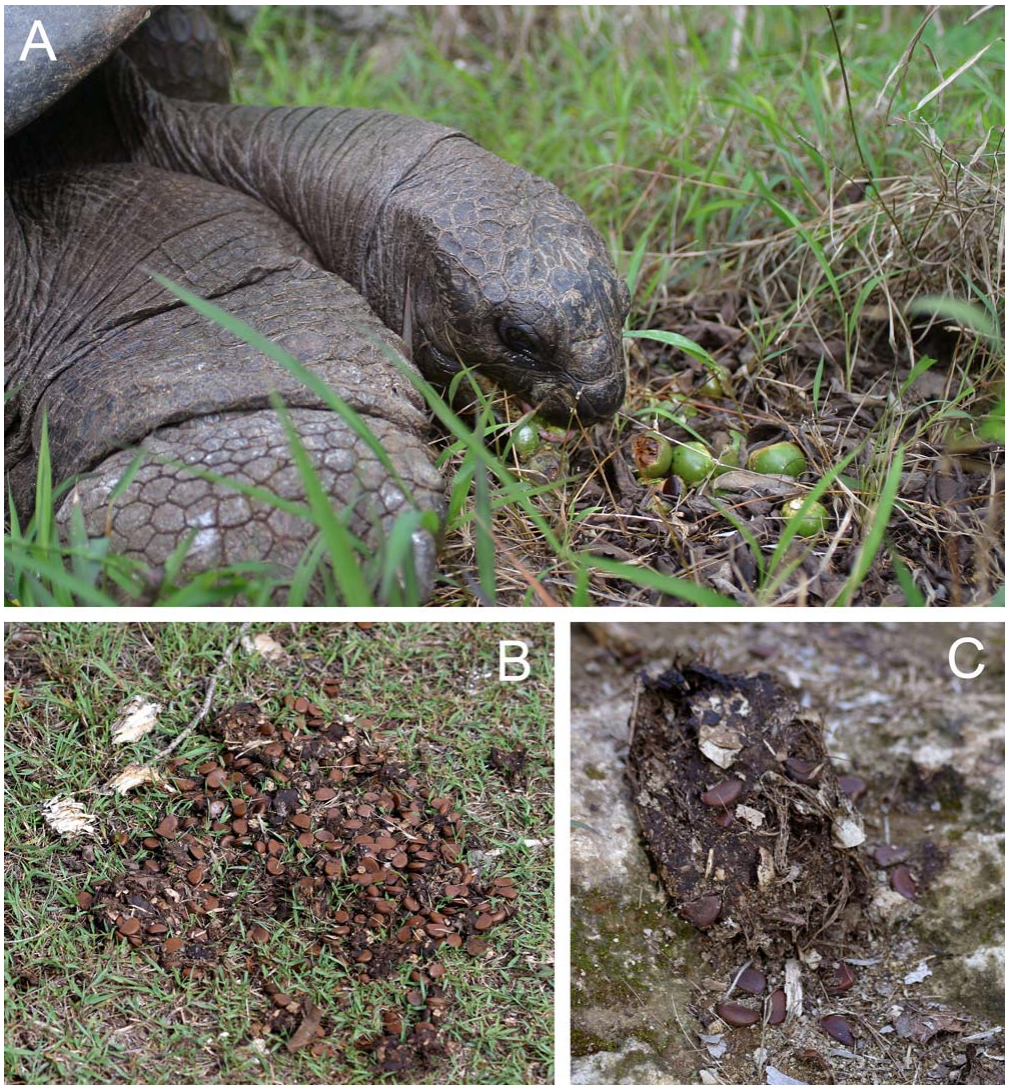
WRIGHT, S.J. (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130: 1-14.

WYSE-JACKSON, P.S., CRONK, Q.C.B. & PARNELL, J.A.N. (1988) Notes on the regeneration of two rare Mauritian endemic trees. *Tropical Ecology* 29: 98-106.

## APPENDIX FIGURES




**APPENDIX FIGURE 1.** (A) Developing fruits on the lower ~50 cm of a *Syzygium mamillatum* tree. (B) Ripe fruits attached to the trunk. Note the foremost fruit has split open, releasing a fermented smell. (C) A 'ball' of four seeds from one fruit with the pulp removed. (D) Germinating seed. Note the clear line between the two green cotyledons. (E) Giant Aldabra tortoise feeding on *S. mamillatum* fruits. (F) Seeds with and without the slimy, fibrous endocarp. (G) Seed fragments after tortoise gut-passage. Fragments were most often found as whole cotyledons. Note how some cotyledons are still green on the side that faced the other cotyledon, suggesting that they did not break apart until late in the gut passage. (H) A caged patch of seeds. (I) A patch of seedlings.




**APPENDIX FIGURE 2.** (A) In the nature reserve on the offshore Mauritian island Ile aux Aigrettes, released free-roaming giant Aldabran tortoises *Aldabrachelys gigantea* eat fruits of the endangered endemic ebony *Diospyros egrettarum*. (B, C) In the fruiting season, one tortoise turd can contain up to several hundred seeds, the vast majority of which have survived the gut passage unscathed. Formerly restricted to one small patch on the 25-ha island, young ebony seedlings can now be found widespread across much of the island, attesting to the potential of *A. gigantea* as ecological analogues for the two extinct Mauritian giant tortoises.

# CHAPTER 6

## The critically endangered Mauritian endemic plant *Roussea simplex* (Rousseaceae): geckos as pollinators and seed dispersers

"...the geckos were busily engaged opening the flowers with their snouts and plunging their heads inside; it was noticed that the flowers were particularly rich in nectar".

–Vinson & Vinson, 1969, observing *Phelsuma ornata* geckos

## ABSTRACT

*Roussea simplex* is the sole member of the enigmatic endemic family Rousseaceae from Mauritius. It occupies a basal position in the Asterales, and its restricted occurrence on an isolated, young volcanic island makes the study of its ecology important for our understanding of the evolutionary ecology and biogeography of Asterales. *Roussea simplex* is critically endangered with 85–90 known remaining individuals, and active conservation management is urgently required. We documented the flowering and fruiting phenology, and studied the pollination and seed dispersal ecology of *R. simplex* in two populations. Our results confirmed preliminary observations suggesting endemic diurnal *Phelsuma cepediana* geckos acting as pollinators. These geckos were also the only animals eating the pulp and dispersing the tiny seeds. In experiments with captive geckos, we confirmed that geckos ingest the seeds and pass them unharmed. This makes *R. simplex* one of the few known plants that utilise the same animal species for both pollination and seed dispersal. However, none of the seeds from fruits or gut-passed seeds germinated, possibly because of fungal attack. We provide detailed data for the flowering and fruiting phenology, and the pollination and seed dispersal biology of *R. simplex*. However, we also highlight the large gap that remains in our understanding of the germination and regeneration of *R. simplex*, and conservation management must address this in the near future.

## INTRODUCTION

*Roussea simplex* Sm. is the sole member of the enigmatic endemic family Rousseaceae from the island of Mauritius in the Indian Ocean. Today, *R. simplex* is critically endangered, occurring only in few populations with very few individuals, totalling some 85–90 known adult individual plants (Fig. 1; Table 1; Friedmann 1988; Scott 1997). However, it was once a widespread and locally common species in wet high-altitude forests in Mauritius. So much so, that Vaughan and Wiehe (1937, p. 314) remarked that in some places, "...an extremely thick canopy of woody lianes (*Roussea simplex* [...] develops about 4–6 m. above ground-level, causing such dense shade that both terrestrial and epiphytic plants are practically excluded". The taxonomy of *R. simplex* has been debated ever since its first description in 1789. Recently, using molecular data, Lundberg (2001) circumscribed Rousseaceae to include a larger monophyletic clade together with Carpodetaceae. Carpodetaceae comprises three small genera, *Carpodetus* (two species, *C. arboreus* in New Guinea and the Solomon Islands, and *C. serratus* in New Zealand), *Cuttsia* (one species, *C. viburnea*, in Australia), and *Abrophyllum* (two species, *A. microcarpum* and *A. ornans* in Australia) (Gustafsson & Bremer 1997; Gustafsson in press). However, the monophyly of each of the two resulting subfamilies is as well supported as the larger clade, and Koontz *et al.* (in press) suggest maintaining them as two separate families. The peculiar biogeography of Rousseaceae sensu lato is evident, with *R. simplex* occurring on Mauritius and the genera *Carpodetus*, *Cuttsia* and *Abrophyllum* in Carpodetaceae from much further east in eastern Australia, New Guinea, and New Zealand. While Rousseaceae s.l. has a possible sister relationship with Campanuleaceae, the phylogenetic split between the large basal clade including Rousseaceae s.l. and Campanulaceae, and the rest of Asterales is only weakly supported (Lundberg & Bremer 2003). Nevertheless, the tentative basal position of *R. simplex* in the Asterales, combined with its restricted occurrence on a young volcanic island, gives *R. simplex* a key role in understanding the evolution and biogeography of Asterales (Bremer & Gustafsson 1997; Lundberg 2001).

In our study, we investigated the pollination and seed dispersal biology of *R. simplex*. These are two important processes in the life cycle of plants, and in many tropical plants they are mediated by animals (Howe & Smallwood 1982; Bawa 1990). Such a study of the pollination and seed dispersal biology of *R. simplex* is important not only for conservation management, but also for understanding the evolutionary ecology of Asterales as a whole. Furthermore, knowledge of the seed dispersal biology of *R. simplex*

will enhance our understanding of how *Roussea* originally may have arrived in Mauritius, and is thus of great importance in disentangling the puzzling biogeography of Rousseaceae s.l.

Little is known about the pollination biology of *R. simplex*. The flowers are visited by several animal species, including the endemic nectarivorous passerine *Zosterops mauritianus*, the endemic day-gecko *Phelsuma cepediana*, several small Diptera, the introduced Hymenopterans *Apis mellifera* (honey bee), *Polistes hebraeus* (wasp), and *Technomyrmex albipes* (ant), and a native Lepidoptera *Henotesia narcissus* (Hansen 2005, pers. obs.; C.N. Kaiser, unpubl. data). Apart from the geckos, the birds and the ants, all other arthropod flower visitors have only been observed occasionally, and never observed receiving a pollen load by touching the anthers. The flies feed on the nectar, but are too small to touch any of the reproductive surfaces upon entering the flower. The butterflies were never seen to enter the flowers, but probed their long probosces into the nectar from the side, between two petals. The large wasps were only seen entering female phase flowers, and the honey bees predominantly entered female phase flowers as well, being mostly unable to squeeze into the narrow male phase flowers. Honey bees were never observed touching the anthers. Previously, Hansen (2005) showed that although the birds entered the flowers in a way that could result in pollen transfer, the feathers on their foreheads got bedraggled with a mixture of sticky pollen and nectar that was unlikely to effect any transfer onto stigmas. The influence of the introduced and invasive ant *T. albipes* on the pollination and seed dispersal interactions of *R. simplex* is the focus of Chapter 7, and will not be dealt with in detail in this chapter. No previous studies have investigated the seed dispersal biology of *R. simplex*, but preliminary observations in 2004 showed that the *P. cepediana* geckos foraged on its fruits.

Here, we focus on the role of the gecko *P. cepediana* in the pollination and seed dispersal biology of *R. simplex*, and speculate on the role of other potential seed dispersers. Our aim is threefold: Firstly, to investigate and document the pollination and seed dispersal biology of *R. simplex*. Secondly, to address the potential implications of our findings for a wider understanding of the biogeography and evolutionary ecology of the basal Asterales. Thirdly, to suggest appropriate conservation measures for *R. simplex* based on our findings.

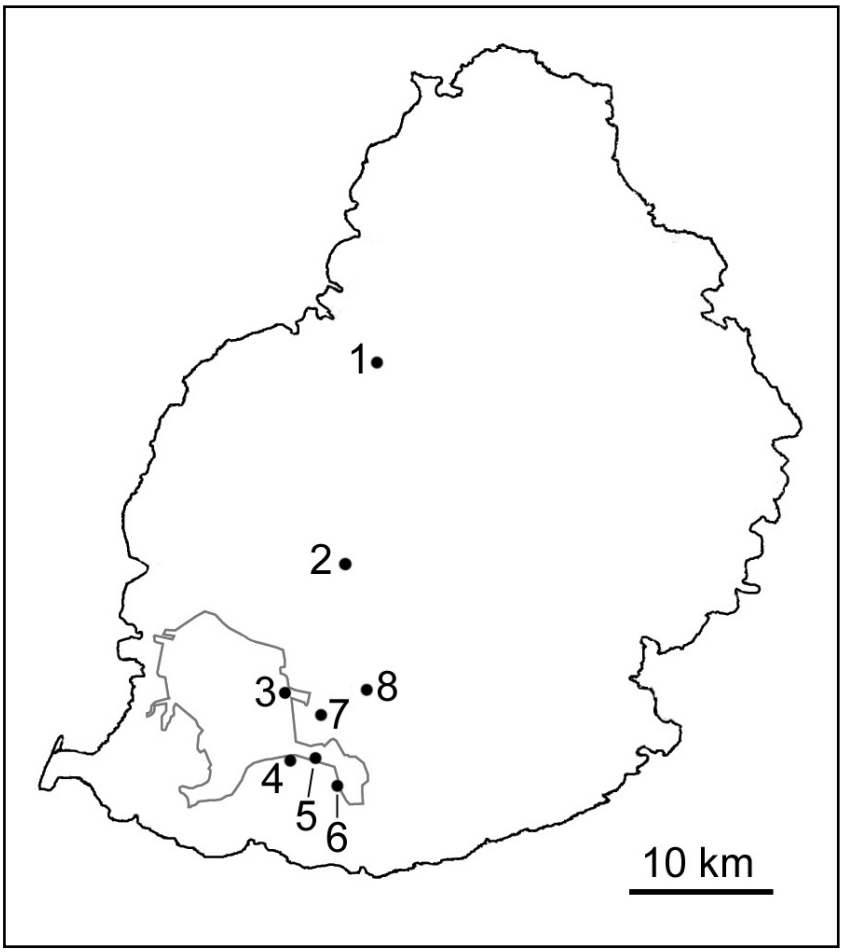



**FIGURE 1**. Map of Mauritius showing the locations of *Roussea simplex* populations, with the Black River Gorges National Park outlined in grey. The numbers indicate the location of the populations, as follows: 1) Pouce; 2) Trou aux Cerfs; 3) Pétrin; 4) Bassin Blanc; 5) Pigeon Wood; 6) Piton Savanne; 7) Kanaka Crater, 8) Grand Bassin.

**TABLE 1.** Populations of *Roussea simplex* in Mauritius, with the numbers of plants found during surveys in 2003 and 2004, and the current conservation status of the habitats where they occur.

| Population | Plants | Population habitat conservation status |
|---|---|---|
| Le Pouce | 29 | Forestry Service Nature Reserve, degraded native forest |
| Trou aux Cerfs | ~5[1] | Exotic pine forest |
| Pétrin | 3 | Conservation Management Area, inside National Park |
| Bassin Blanc | 17 | Highly degraded native forest, outside National Park |
| Pigeon Wood | 6 | Highly degraded native forest, inside National Park |
| Piton Savanne | 23 | Highly degraded native forest, outside National Park |
| Kanaka Crater | 0?[2] | Highly degraded native forest, outside National Park |
| Grand Bassin | 7 | Highly degraded native forest, outside National Park |

[1] A 'handful' of plants were seen on the southern edge of the crater in 2005 (V.F. Florens pers. comm.).
[2] Last recorded from here in 1932; despite repeated surveys we found no plants in 2003 or 2004.

## MATERIALS AND METHODS

### *Study species*

*Roussea simplex* is a climbing shrub, covering areas of up to 4 × 5 m, and often leaning onto other plants, sometimes strangling them. It has opposite, large (7–12 × 3–3.5 cm), thick and rigid leaves with serrate-glandular margins. Flowers are yellow to orange and large (~2.5 cm corolla diameter and length), borne singly in the leaf axis. The peduncles are short (1–1.5 cm), thick (~5 mm) and rigid, with flowers typically pointing downwards but they can also be horizontal or even upward-facing. Flowers are protandrous, with large, thick stamens and anthers. Anthers split open lenghtwise and secrete a sticky, slimy pollen-substance that adheres to any surface touching it. The pollen grains are spheroid, ~30 μm in diameter with a smooth surface and 5–6 pores (Hansen 2005; Koontz *et al.* in press). Later, stamens fall off to reveal the thick style and the large, circular stigma (Fig. 2). Flowers have a weak, sweet and slightly fermented smell, and produce copious amounts of nectar (Hansen 2005).

*Phelsuma cepediana* is one of five endemic *Phelsuma* species in Mauritius. It is a medium-sized gecko (max snout-vent lengths: males = 58 mm, females = 49 mm), found on the wet central plateau and southern highland, with a generalised diet of invertebrates, nectar, and fruit (Vinson & Vinson 1969; Vinson 1976). *Phelsuma* geckos have excellent colour vision (Taniguchi *et al.* 1999) and acute olfactory perception (Schwenk 1993). At Pétrin and elsewhere in Mauritius, *P. cepediana* has been observed visiting the flowers of many different native and endemic plant species, serving as efficient pollinators of at least some of them (Chapter 3; DMH pers. obs.; Kaiser 2006).

### *Study sites*

Mauritius, one of the three Mascarene Islands, is an 8 my old volcanic island of 1865 $km^2$ in size, located approximately 800 km east of Madagascar. Today, less than 2% of original native vegetation remains in Mauritius, most of which lies within the Black River Gorges National Park (Fig. 1). Much of the remaining native vegetation is heavily degraded by a few invasive plant species, especially *Psidium cattleianum* and *Syzygium jambos* (Myrtaceae), *Ligustrum robustum* (Oleaceae), *Hiptage benghalensis* (Malphigiaceae) and several shrubby understory species, e.g. *Clidemia hirta* (Melastomataceae), *Rubus alceifolius* (Rosaceae), and *Ardisia crenata* (Myrsinaceae).

During surveys in 2003 and 2004, we searched most of the known sites of *R. simplex* and recorded the number of individual plants (Fig. 1; Table 1). We selected Pétrin

and Bassin Blanc as our main study populations for observations and experiments, but will supplement our results and discussion with data and observations from the other populations. Pétrin, located in the Black River Gorges National Park, is one of the largest remaining areas of upland heath in Mauritius. This habitat type is found on old, weathered lava without much topsoil, and is dominated by a low, shrubby vegetation of 1–2 m in height with a few emergent trees of 3–5 m in height. In Pétrin, there is a 6.2 ha fenced and weeded conservation management area (CMA), and there are three large *R. simplex* plants within the CMA (hereafter, 'Pétrin' refers to the CMA only). Bassin Blanc is a crater lake, located on the southern slopes of Mauritius. Around the crater itself there is some relatively good native forest left, but the population of *R. simplex* is found a few hundred metres north, on a heavily degraded, steep slope with a few native trees of 5–8 m in height, and a dense shrubby vegetation that is dominated by the invasive *P. cattleianum*. Here we found 17 adult *R. simplex* plants, all growing within an area of ~0.5 ha.

*Phenology data*

We collected data on flowering phenology to supplement the preliminary data presented in Hansen (2005) and investigated several aspects of fruiting phenology. To investigate anthesis patterns, we marked buds that were about to open, and recorded the sexual phase of the flowers daily until wilting. For nectar, we measured the standing crop and sugar concentration in buds that were about to open (by prying them open with forceps), and in male and female phase flowers at noon. We also measured diel nectar production, roughly split up into diurnal and nocturnal nectar production, for both the male and female phase flowers. We either emptied flowers of nectar between 08:00–09:00 h, bagged them, and measured nectar volume and concentration again between 16:00–17:00 h (diurnal production), or emptied flowers between 16:00–17:00 h and measured nectar volume and concentration between 08:00–09:00 h the following day (nocturnal production). We did three replications of all the above measurements on each of three plants in Pétrin and on three plants at Bassin Blanc. Measurements were analysed with ANOVAs. To analyse sugar composition, we collected six 5 µL samples of nectar on filter paper from three male and three female phase flowers, one of each from the three plants in Pétrin. These were taken to Switzerland and analysed using HPLC.

To record fruiting phenology, we marked three fruits that were just about to open (see Fig. 2G) on each of three plants in Pétrin and on three plants at Bassin Blanc and recorded daily whether they still contained fresh pulp.

*Hand pollination experiments and seed set*

In November 2004, we bagged buds and applied one of three different treatments: autogamy (selfing), geitonogamy (self-compatibility) or xenogamy (outcrossing). For autogamy, we simply left bagged flowers untouched throughout anthesis. For geitonogamy and xenogamy, opening buds were emasculated by cutting off the anthers. Then, upon flowers entering female phase (anthers falling off, stigma swelling and changing colour from green to pale green or cream; Fig. 2E, Fig. 3C,D), we transferred pollen from a freshly opened male flower on the same plant or pollen from another plant in the same population, respectively. In total, we set up two flowers of each treatment on the three plants in Pétrin, and two flowers of each treatment on five plants at Bassin Blanc. We also investigated natural levels of seed set in flowers to which pollinators had had access. For this, we marked late female phase flowers without an infestation of invasive *Technomyrmex albipes* ants, as these were unlikely to have had a prior infestation of ants during male or early female phase (see Chapter 7). We marked three flowers on each of the three plants in Pétrin and three plants at Bassin Blanc. Developing fruits from all the treatments and the open-pollinated control flowers were harvested, and seed set scored, in late February 2005. Seed set of the different treatments and open-pollinated fruits were compared with an ANOVA.

To obtain a baseline number against which to compare the seed sets of the experimental and of the naturally pollinated flowers, we counted the numbers of ovules and ovary locules in the ovaries of freshly wilted flowers; three ovaries from three plants in both Pétrin and Bassin Blanc, for a total of 18 ovaries. Both ovules and seeds were counted by cutting ~2 mm cross-sections off the ovaries and estimating the ovules or seeds in groups of five or ten with a dissecting needle under a Nikon 20× magnification field dissecting microscope.

*Flower visitor and frugivore observations*

Flower visitor and frugivore observations were made with 10 × 32 mm Leica binoculars from a distance of 4–5 m, with the sitting observer either mostly covered by vegetation or by a 1 × 3 m lightweight camouflage net draped over the head and shoulders. After setting up the observation post, the observer remained as motionless and quiet as possible for 20 min before starting to record flower visitation and fruit feeding by geckos and other animals, to allow nearby animals to become accustomed to the presence of a human.

Observation periods were either 60 or 90 min for flower observations, and 90 or 120 min for fruit observations. For flowers, we observed 4–8 flowers in each period, with equal numbers of male and female phase flowers. For fruits, we observed 3–5 fruits in each period. Preliminary observations indicated that, depending on sex and size, geckos could behave differently at flowers and fruits of *R. simplex*. Adult male *P. cepediana* geckos are easily identified as such, being large and typically having bright blue or turquoise lower backs and tails (Fig. 3A,F), whereas female and juvenile geckos are much harder to distinguish, being drab brown and green in colour (Fig. 3D). Therefore, we divided observed geckos into two groups: (1) adult males and (2) females plus juveniles. We recorded both the number of visits per observation period, and the length of the visit. A visit started when a gecko first probed a flower or started feeding at a fruit, and ended when the animal left the flower or fruit. We only observed flowers and fruits without any congregations of *Technomyrmex albipes* ants, because these ants alter the behaviour of the geckos dramatically (see Chapter 7). We analysed the gecko visitation rates at flowers and fruits with a linear mixed-effects model (LMM), with gecko sex/age, flowers/fruits, and study population as main effects and observed plant ID as a random effect. Similarly, we investigated duration of visits with a LMM, with gecko sex/age, flowers/fruits, and study population as main effects and observed plant ID nested in observation period as a random effect.

Lastly, flower visitation rate of *P. cepediana* is known to be affected by habitat structure at a small spatial scale. In particular, dense patches of endemic *Pandanus* (Pandanaceae) species are favoured microhabitats for the geckos, as it may protect them from attacks by their main native predator, the Mauritius kestrel *Falco punctatus* (Nicoll *et al.* 2003). Hence, flowering plants close to these patches are likely to receive more visits by geckos than plants away from such *Pandanus* patches (see Chapter 3). In Pétrin, two of the three *R. simplex* plants were growing at the edges of large *Pandanus* patches, while the last plant was more isolated, with > 15 m to the closest *Pandanus* patch. We therefore additionally analysed visitation rates at flowers and fruits of *R. simplex* at the three plants in Pétrin in relation to proximity to *Pandanus* patches (close or away) with ANOVAs, and duration of visits in relation to proximity to *Pandanus* patches with LMMs with observation period as a random effect.

*Feeding and germination experiments*

The observations at the fruits showed that only *P. cepediana* geckos were feeding on the pulp of *R. simplex* fruits (see Results). Therefore, we experimentally investigated the effect of gecko gut-passage on the seeds. For this experiment, we used three adult *P. cepediana* geckos, two males and a female, caught with a slipknot noose on a telescopic rod in the vegetation around Brise Fér field station between January 31–February 2 2005. The geckos were kept in two 40 × 40 × 60 cm cages, made out of plywood (1.25 cm thick, bottom, back and right side) and fine wire mesh (approximately 2 × 2 mm mesh, top, front and left side). A door (20 × 40 cm) in the lower front provided easy access. The largest male was kept in one cage, while the female and the smaller male were kept together in the other cage. The cages were kept in partly shaded conditions, with sun early and late in the day, and each cage had two branches and several *Pandanus* leaves forming a tent-shaped hideout in one corner. The geckos were kept for approximately one week to acclimatise to the new conditions and had access to water and baby-food (fruit varieties) *ad libitum*. Twice a week they were fed 10–15 live Muscoid flies.

Two days before the feeding experiments started, the baby food was taken out of the cages. Ripe *R. simplex* fruits with a large, fresh mass of pulp with seeds were taken from the three plants in Pétrin CMA and fed to the geckos three times, on February 7, February 9, and February 11. Fruits were attached to the wire mesh on the left sides of the cage between 08:00–10:00 h, and removed again between 14:00–16:00 h. Within 20–40 min of attaching the fruits, the geckos were seen feeding on the fruit pulp. At the same time that the fruits were attached, we covered the entire floor of each cage with a piece of black plastic to facilitate recovery of the semi-fluid gecko droppings. We checked the cages for gecko droppings in the evenings around 18:00–19:00 h and in the mornings around 08:00–09:00 h from February 7 to February 12. Any seeds found were extracted from the dropping, examined under the dissecting microscope and put on moist cotton wool in Petri dishes with roughly 1.5 cm between each seed. We also set up two Petri dishes with seeds that had been manually extracted from ripe pulp. Each of these contained 10 seeds from one fruit from three different plants, for a total of 30 seeds in each dish. One dish contained seeds from the three plants in Pétrin, and the other contained seeds from fruits of three random plants at Bassin Blanc. Lastly, we set up two dishes with small lumps of ripe pulp containing approximately 10 seeds each, from the same fruits and sites as above. Each of these dishes had five lumps of pulp with seeds. All

Petri dishes were kept at the Brise Fér fieldstation near a window, but away from direct sunlight. Seeds were checked daily and the cotton wool was kept moist.

We also attempted to assess seed germination in the field by putting out seeds in three sites in moist litter beneath *Pandanus* patches at Pétrin. However, the seeds are minute and disappeared within few days from all places within 2–5 days, usually after heavy rains, and could not be found again.

All statistical analyses were done with R.2.3.1 (R Development Core Team 2006).

## RESULTS

*Flowering and fruiting phenology*

At Pétrin and Bassin Blanc, flowering of *R. simplex* started in September 2004 and lasted until late January 2005, while ripe fruits were available from early to mid-January to mid-May. Individual plants flowered during most of the flowering period and could have both flowers and ripe fruits simultaneously in the temporal overlap between flowering and fruiting. The temporal sequence from bud to ripe fruit is illustrated and annotated in Fig. 2. Flowers were open for a total of 6–8 days (7.1 ± 0.2 days; N = 18 flowers; all means ± 1SE), with male phase lasting 2–5 days (3.3 ± 0.2 days; N = 18 flowers), and female phase lasting 3–5 days (3.8 ± 0.2 days; N = 18 flowers). Fruits presented pulp for a total of 4–7 days (5.4 ± 0.2 days; N = 18 fruits). Flowers had very large standing crops of nectar (Table 2), which was hexose-dominated with only trace amounts of sucrose (glucose = 52.8 ± 1.6%, fructose = 47.2 ± 1.6%, N = 6). There were no significant effects of population or flower sexual phase on volume or concentration of nectar standing crop (all P-values > 0.1).

Nectar production probably started at least 1–2 days before anthesis, as large unopened buds contained very large amounts of nectar of up to just above 1 mL (Table 2). During anthesis, more nectar was produced over night than during the day ($F_{1,68} = 6.99$, $P = 0.010$) and male phase flowers produced more nectar than female phase flowers ($F_{1,68} = 8.69$, $P = 0.004$). There were no significant differences in nectar production between Pétrin and Bassin Blanc ($F_{1,68} = 0.042$, $P = 0.838$). For concentration of nectar produced, there were no significant effects of production time, population or flower sexual phase (all P-values > 0.2).

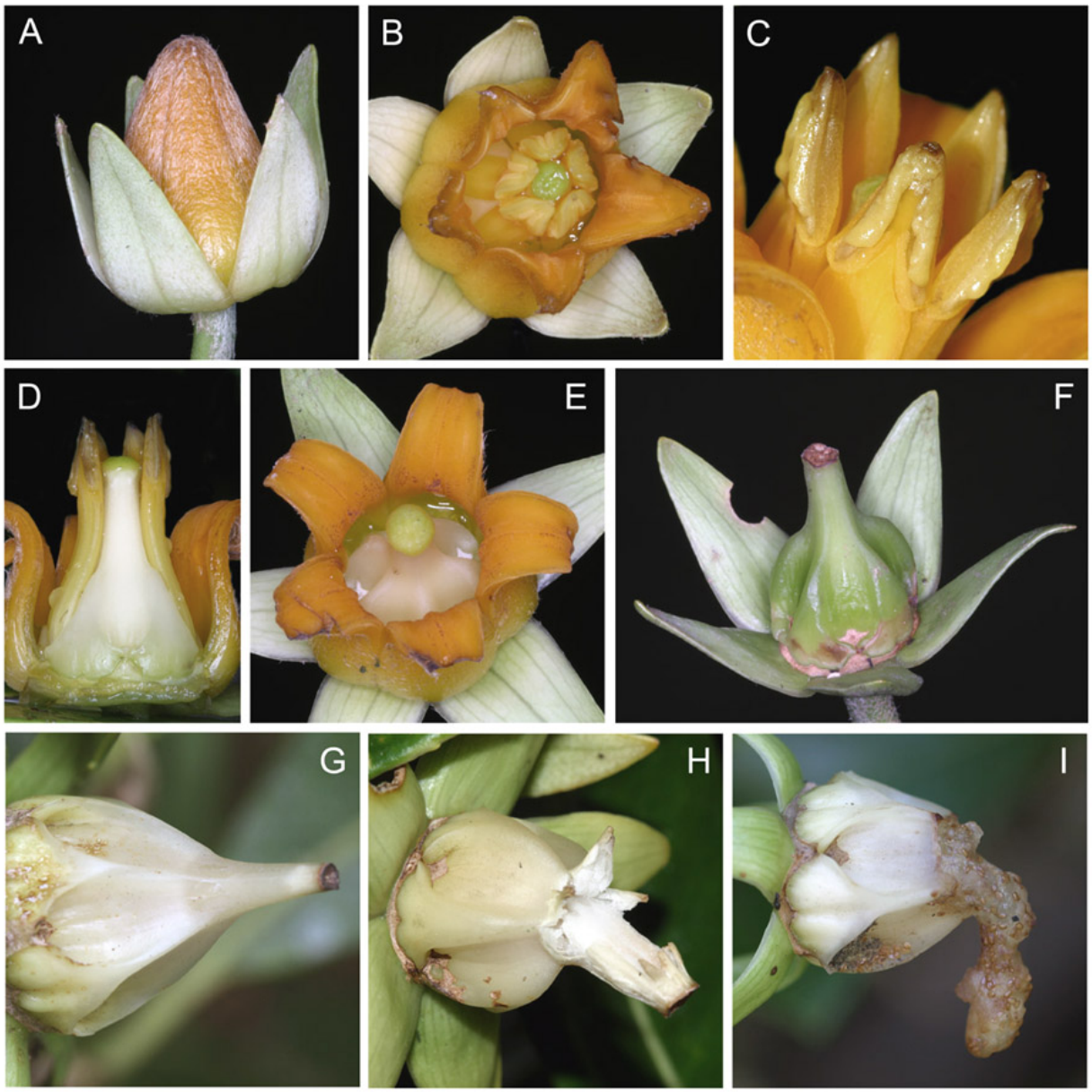



**FIGURE 2.** Flowering and fruiting phenology of *Roussea simplex*. (A) Bud, ready to open. (B) Young male phase flower, anthers not dehisced yet. Note the green stigma, and the reflection in the plentiful nectar at the base of the flower cup. (C) Close-up of male phase flower, showing the slimy pollen excreted in long 'sausages' along slits in the anthers. (D) Cross-section of male phase flower, with one anther removed to show pale ovary and thick style. The entire gynoecium is very rigid and hard (E) Female phase flower; all stamens dropped off, style now swollen and pale yellow (cf. Fig. 3C,D). Note the still plentiful nectar in the flower cup. (F) Developing fruit, ca. one month after flower wilted. The stigma dries and falls off after ca. one week, and the developing fruit swells and turns dark green, remaining hard. (G) Ripe fruit, about to burst open. When ripe, fruits turn pale cream, but is still very hard except for the style which turns soft. (H) The style bursts and the central column of the ovary is pushed out. (I) The slimy pulp with the embedded small seeds is then slowly secreted during several days. Photo (H) by C. N. Kaiser, all other photos by DMH.

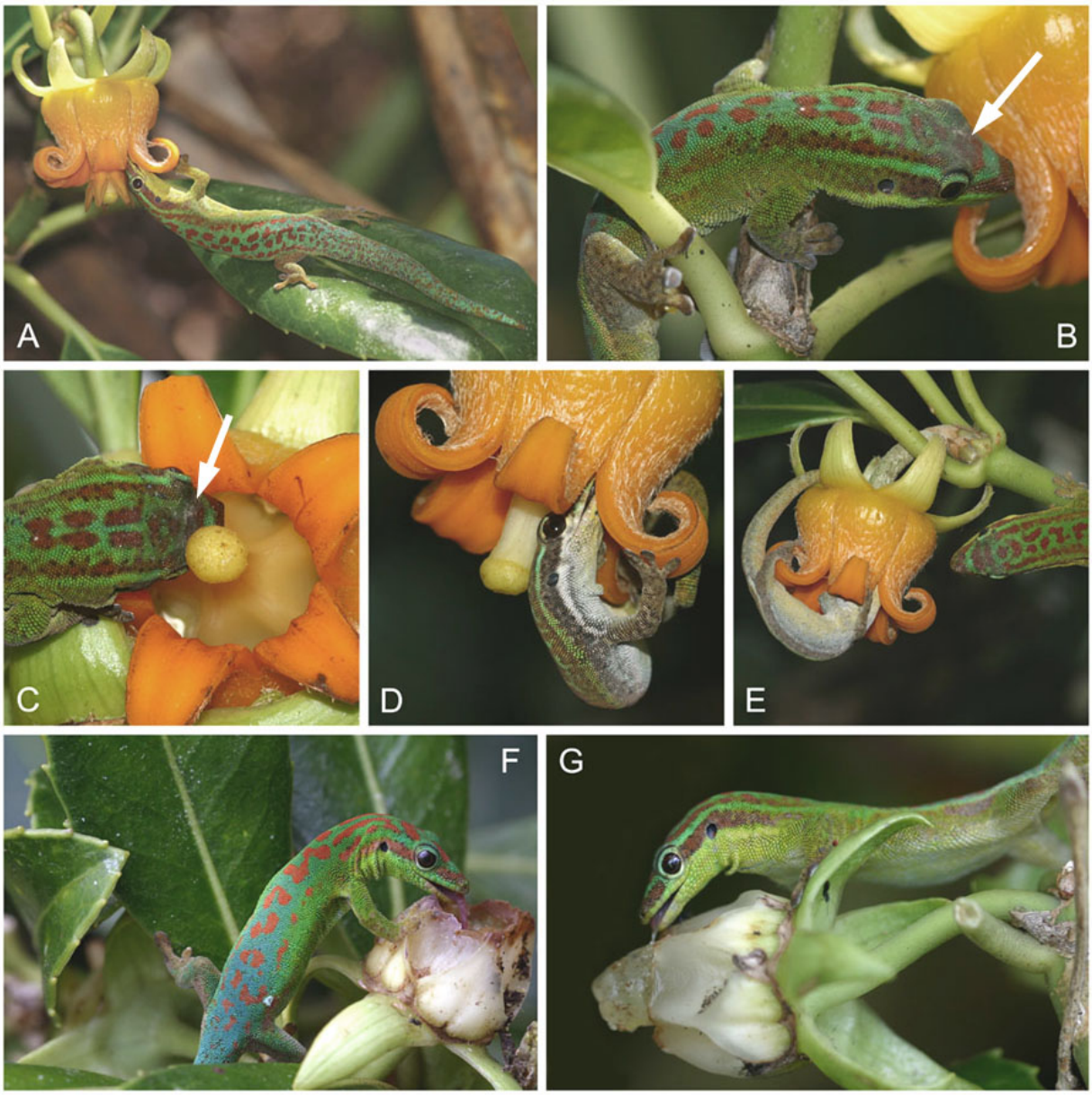



**FIGURE 3.** *Phelsuma cepediana* geckos foraging at flowers and fruits of *Roussea simplex*. (A) Adult male gecko visiting a male phase flower, getting a smear of the slimy pollen substance (cf. Fig 2C) on the forehead. (B,C) Adult male gecko with the characteristic smear of pollen on the forehead (arrows), approaching and entering a female phase flower, where some of the pollen is then deposited on the stigma. (D) Female or juvenile gecko visiting a female phase flower. (E) If there is only little nectar left in a flower, the smaller female and juvenile geckos must insert their head deeper into the flower, leaving themselves vulnerable to harassment or attack by large males. (F, G) Adult male geckos foraging at fruits, using a mixture of licking and chewing at the pulp, swallowing the tiny seeds in the process.

**TABLE 2.** Nectar phenology of *Roussea simplex* buds and flowers. There were no significant differences between plants from Pétrin and Bassin Blanc; hence measurements are pooled. Sample size for all values is 18.

| | | Volume (µL) | | Concentration (%) | |
|---|---|---|---|---|---|
| | | Range | Mean ± 1SE | Range | Mean ± 1SE |
| Bud | Standing crop | 120–1185 | 511.3 ± 67.2 | 6.5–11.5 | 8.9 ± 0.4 |
| Male phase | Standing crop | 21–510 | 195.7 ± 32.8 | 7.5–12.5 | 9.9 ± 0.3 |
| | Diurnal production | 67–305 | 190.1 ± 16.5 | 6.5–14 | 9.8 ± 0.4 |
| | Nocturnal production | 110–380 | 234.2 ± 15.6 | 7.5–14 | 10 ± 0.4 |
| Female phase | Standing crop | 42–335 | 163 ± 19.1 | 7.5–13 | 10.7 ± 0.4 |
| | Diurnal production | 70–285 | 155.5 ± 13.2 | 6.5–12.5 | 9.5 ± 0.4 |
| | Nocturnal production | 130–275 | 185.8 ± 9.95 | 7.5–13 | 10.1 ± 0.3 |

*Hand pollination experiments and seed set*

The ovaries of *R. simplex* flowers contained *ca.* 2100–3300 ovules (2572 ± 79 ovules) in 5–10 ovary locules (6.5 ± 0.3 locules; N = 18 ovaries). We had to harvest the experimental fruits before they ripened and opened (see Fig. 2G-I) to count all seeds. None of the flowers in the autogamy treatment produced any seeds. Geitonogamously pollinated flowers produced fruits with an average of 399 ± 29 seeds (~16% seed set, N = 16 fruits) and xenogamous fruits produced slightly more seeds, with an average of 476 ± 47 seeds (~19% seed set, N = 16 fruits). The developing fruits from naturally pollinated flowers contained an average of 505 ± 52 seeds (~20% seed set; N = 18 fruits). Numbers of seeds in these latter three categories were not significantly different from one another ($F_{2,47}$ = 1.51, P = 0.231). Seeds of ripe fruits were around 0.7–1.1 mm long, 0.5–0.8 mm wide and ~0.3 mm thick, with a thin, semi-hard light-brown endocarp (Fig. 2I), and white endosperm. A puzzling result was that while the autogamy treatment did not result in any seeds being produced, fruits and pulp still developed like in fertilised fruits. In pilot experiments in 2003, autogamy resulted in fruits that ripened and opened like normal fruits, but whose pulp contained no seeds (unpubl. data).

*Flower visitor and frugivore observations*

At the flowers, we observed *P. cepediana* geckos, *Z. mauritianus* birds, and several of the previously observed invertebrates: honey bees, flies, the ant *T. albipes* and the butterfly *Henotesia narcissus*. We consider none of the invertebrates nor the bird capable of transferring *R. simplex* pollen. We therefore only give detailed results for *P. cepediana*

flower visitation. At ripe fruits we only observed *P. cepediana* geckos feeding on the pulp. Several frugivorous birds were observed in the vicinity of fruiting plants (*Z. mauritianus* and the introduced red-whiskered bulbul *Pycnonotus jocosus*) but they did not show any interest in these fruits.

Only one gecko at a time visited a particular flower or fruit. If a gecko was already foraging at a flower or fruit, another approaching gecko of a smaller size would usually wait at a minimum distance of 30–40 cm until the foraging gecko had finished and left, unless it was a large adult male. Generally, if the approaching gecko was a large adult male a foraging, smaller gecko would be displaced without a fight. Occasionally, equal-sized geckos would display threat gestures towards each other or enter a very brief fight that would end with one of them rapidly retreating. During most flower visits, geckos inserted their heads into the corolla with the forehead towards the centre of the flower, thus either receiving a slimy smear of pollen at male phase flowers (Fig. 3A), or depositing pollen on the stigma at female phase flowers (Fig. 3C,D). We often saw geckos with a large part of the forehead, neck and upper back covered in a thin layer of the slimy pollen-substance (Fig. 3B,C). Foraging at fruits, geckos usually used a mixture of licking and eating lumps of pulp with seeds (Fig. 3F,G), and otherwise behaved in the same way as outlined above for nectar feeding at flowers.

The analyses of the visitation rates demonstrated that *P. cepediana* visits were significantly more frequent to flowers than to fruits (Fig. 4A,B; $F_{1,98} = 4.75$, $P = 0.032$), and that there was a significant effect of gecko sex/age, with adult male geckos visiting more frequently than females and juveniles (Fig. 4A,B; $F_{1,98} = 41.0$, $P < 0.001$). There was a marginally significant difference between study populations, with slightly more visits per hour in Pétrin than at Bassin Blanc (Fig. 4A,B; $F_{1,6} = 5.39$, $P = 0.059$). All interactions between main effects were non-significant, and values given here are from a minimum adequate model with only main effects fitted. Duration of visits varied as well, but contrary to visitation rate, the geckos foraged longer at fruits than at flowers (Fig. 4C,D; $F_{1,233} = 127.8$, $P < 0.001$), and they foraged longer at Bassin Blanc than in Pétrin (Fig. 4C,D; $F_{1,233} = 4.05$, $P = 0.045$). However, the significant effect of gecko sex/age was similar to that observed for foraging at flowers, with adult males foraging longer than females and juveniles (Fig. 4C,D; $F_{1,233} = 46.7$, $P < 0.001$). All interactions between main effects were non-significant, and values given here are from a minimum adequate model with only main effects fitted.

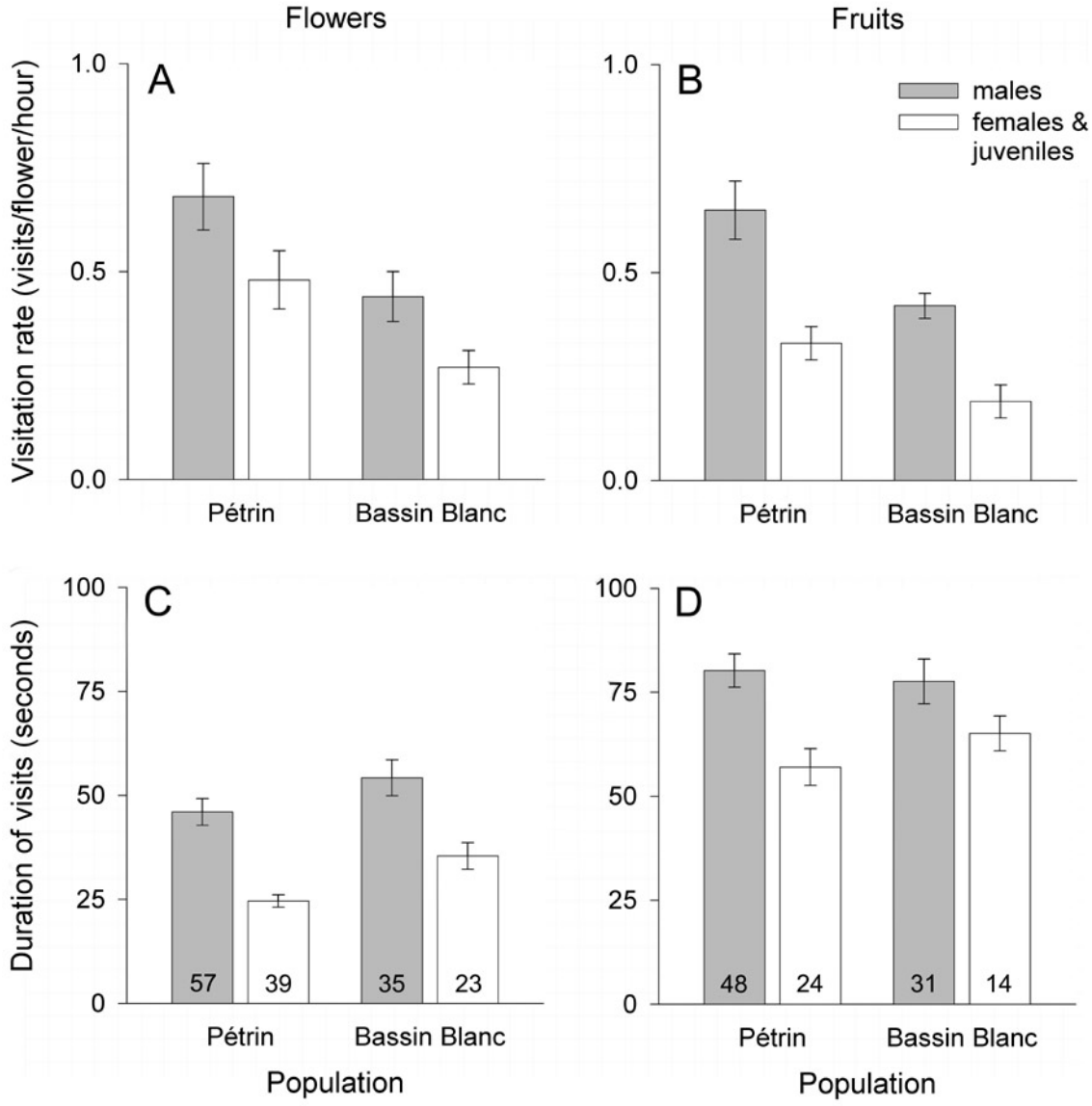



**FIGURE 4.** Visitation rates (A, B) and duration of visits (C, D) of adult male and female/juvenile *Phelsuma cepediana* geckos at flowers and fruits of *Roussea simplex* in the two study populations, Pétrin and Bassin Blanc. Bars are mean values ± 1SE. Sample size for bars in (A) = 15 observation periods, in (B) = 12 observation periods. Sample sizes (number of visits) for bars in (C) and (D) are shown in each bar.

In Pétrin there were significant effects of proximity to *Pandanus* patches on gecko visitation rates for both flowers (close: 1.44 ± 0.13 visits/flower/hour; away: 0.64 ± 0.09 visits/flower/hour; $F_{1,13} = 16.7$, $P = 0.001$) and fruits (close: 1.15 ± 0.11 visits/flower/hour; away: 0.63 ± 0.06 visits/flower/hour; $F_{1,10} = 9.46$, $P = 0.012$). There were no significant interactions between proximity to *Pandanus* and gecko sex/age for foraging at either fruit or flowers (both $P > 0.50$), hence reported results are for models with adult male and female/juvenile visitation rates pooled within observation periods. However, there were no significant effects of proximity to *Pandanus* patches on duration of visits for flowers ($F_{1,80}$

= 0.02, P = 0.372) or for fruits ($F_{1,56}$ = 0.23, P = 0.634). There were no significant interactions between proximity to *Pandanus* and gecko sex/age (both P > 0.30), hence the reported results are for models with duration of adult male and female/juvenile visits pooled.

*Feeding and germination experiments*

A total of 18 seeds were retrieved from gecko droppings in the cages. Gut-passage time was a few hours or less, as seeds were only found in the evenings after the geckos had been feeding on the fruit pulp during the day. In the gecko droppings, we found only whole seeds with no visible marks or damage to the endocarp.

All the lumps of pulp were attacked by fungi after 3–5 days, and none of the seeds germinated. The Petri dishes with manually depulped seeds and the gut-passed seeds remained free of fungal attack (dark brown or black hyphae clearly visible under dissecting microscope) for 7–12 days after setup, after which time seeds here were also attacked and turned dark brown or black. Single seeds were removed from the dish as soon as they were attacked. The experiments were terminated in late February, when the last remaining seeds were attacked by fungi. Despite staying swollen and looking healthy until attacked by fungi, none of the seeds germinated.

## Discussion

Our results illustrate how *Phelsuma cepediana* geckos are efficient pollinators of *Roussea simplex*, and that the geckos are currently the only animals feeding on the fruits and acting as seed dispersers. Our feeding experiment with captive geckos showed that they are capable of dispersing the tiny seeds unharmed. However, none of the seeds in the experiments germinated, illustrating the large gap that still remains in our understanding of the reproductive biology of *R. simplex*.

*Pollination and seed dispersal*

Geckos accessed flowers and fruits from nearby leaves or branches, or via the short peduncles onto the flowers or fruits themselves, and foraged at flowers and fruits for prolonged periods of time. For flowers, this led to repeated contacts with anthers at male phase flowers, and stigmas at female phase flowers. While it was impossible to see individual seeds being swallowed during the observation periods, close-up observations confirmed that geckos regularly swallowed small lumps of pulp containing seeds. Within

an observed region of a flowering or fruiting *R. simplex* plant (typically covering 0.5–1 $m^2$) we would see only one gecko foraging at any one time. *Phelsuma* geckos in general are aggressive towards each other (Harmon 2005), regardless of sex or size, with the smaller individual quickly disappearing when a larger gecko approaches. This probably explains the behavioural difference between male and female/juvenile geckos, and between foraging at flowers or fruits. A foraging large male is less often challenged by an approaching gecko, and thus forages for longer, while a small gecko is more easily displaced from a flower or a fruit. Such size-dependent dominance patterns are common among lizards (e.g. Lopez & Martin 2001; Aragon *et al.* 2006). This could also explain why we only found an increase in duration of a foraging visit for female/juvenile geckos at fruits compared to visit duration at flowers. Males were usually large enough to literally keep an eye out while foraging at flowers (cf. Fig. 2A), while female/juvenile geckos were often so small that they had to insert more of their bodies into the corolla to reach the nectar, rendering them vulnerable to attack (cf. Fig. 2E) and more likely to frequently stop foraging and assume vigilance for approaching geckos or predators. The difference in visitation rates between study populations is probably due to a lower overall density of geckos at Bassin Blanc (pers. obs.); the vegetation here is very degraded, offering few of the typically favoured retreats of *Phelsuma* geckos (e.g. *Pandanus* patches, palms, old trees with holes; Harmon 2005; pers. obs.). The spatial arrangement of such retreats and other favoured microhabitats of *Phelsuma* geckos is likely to structure their mutualistic interactions with plants, and the strong positive effects of proximity to *Pandanus* patches on gecko visitation rates for both flowers and fruits of *R. simplex* confirm our results from Chapter 3. The large, dense stands of *R. simplex* recorded earlier last century may, by themselves, have provided a favourable microhabitat for geckos, thus attracting and maintaining a population of 'in-house' pollinators and seed dispersers. Moreover, the puzzling result of non-fertilised flowers still producing and secreting normal pulp could be a way for fruiting plants to enhance attractiveness to seed dispersers, especially because in ecological terms the fruits are equivalent to flowers – offering a reward over several days.

Movement patterns of geckos will influence their efficiency as mutualists, and one concern could be that lizard-mediated gene flow is relatively restricted, both in terms of pollen transport and seed dispersal. *Phelsuma ornata* geckos in Mauritius move up to 87 m in a straight line in 29 h, but most recorded movements were much shorter, around 10–20 m within a 24 h period (Nyhagen *et al.* 2001). Male *Phelsuma* geckos are known to be territorial (Nyhagen *et al.* 2001; Harmon 2005; pers. obs.) which may limit their

pollination and seed dispersal efficiency. While territoriality may ensure high levels of geitonogamous self-fertilisation, it could also reduce the incidence of inter-plant cross-fertilisation, and lead to relatively local seed dispersal only. However, smaller males and females/juvenile geckos may travel longer distances, and thus provide a more valuable pollinating and seed dispersing service.

A puzzling floral trait of *R. simplex* is the slimy and sticky pollen substance. When investigating anthers in the field under 20× magnification, the pollen grains in the longitudinal 'sausage' were seen to be embedded in a semi-fluid, pale yellow and sticky substance. One possible function could be to prevent pollen-theft by insects; this would make sense for vertebrate-pollinated long-lived flowers with a protracted male phase. Once, we observed a small fly getting trapped by its legs on the viscid, slimy pollen substance and soon afterwards fall prey to a nearby gecko, and the invasive ant *Technomyrmex albipes* has also been observed getting stuck on the pollen (Chapter 7). Another possibility is that it could be an adaptation to lizard pollination, as it could make more pollen grains adhere to the relatively smooth scales than if the pollen was of the normal dry type. This could function in a parallel way to the findings of Traveset and Sáez (1997), who reported that more pollen grains of *Euphorbia dendroides* were carried on the snouts of the pollinating lizards *Podarcis lilfordi* if the lizard snouts had been in contact with the sticky nectar before brushing against the anthers.

It is also noteworthy that *P. cepediana* probably currently serves as the sole pollinator and seed disperser of *R. simplex*. There are relatively few examples of plants being pollinated and having their seeds dispersed by the same animal species. It is known from some mistletoe species in New Zealand, where the bellbird *Anthornis melanura* provides both services (Kelly *et al.* 2004), and from columnar cacti in South America, where the bat *Glossophaga longirostris* pollinates and disperses the fruits of the three cacti species *Pilosocereus tillianus*, *Stenocereus griseus*, and *Subpilocereus repandus* (Soriano & Ruiz 2002). In Brazil, the epiphyte *Dyssochroma vitidiflorum* (Solanaceae) depends on small bats for both pollination and seed dispersal, even though different species of bats serve as pollinators and seed dispersers, respectively (Sazima *et al.* 2003). *Roussea simplex* is the first known example of a plant having the same lizard species as a pollinator and a seed disperser. It is very likely that there are more such 'double-mutualistic' lizard-plant interactions, especially on oceanic islands where lizards are important pollinators and seed dispersers (Olesen & Valido 2003; Godínez-Álvarez 2004; Valido & Olesen in press). For example, in Mauritius another candidate plant species is the endemic palm

*Latania loddigesii*, where the endemic Telfair's skinks *Leiolopisma telfairii* both visit the flowers (pers. obs.) and ingest the seeds (Pernetta *et al.* 2005).

Lastly, when investigating plant–animal interactions in Mauritius, it is imperative to take the 'ecology of the afterlife' (*sensu* Lawton 1995) into account; i.e., some of the locally extinct or recently extinct animal species could have played a role in the ecological interactions and in the evolution of plant traits of *R. simplex*. In pristine Mauritius, birds or fruitbats may have effected both local and long-distance dispersal events (within the island), while lizards only dispersed the seeds locally. If so, then today there is a gap in the population dynamics of *R. simplex*, as only one of the local dispersers is extant. Similarly, a once widespread and locally common proliferously nectar-producing plant, such as *R. simplex*, may have been important for native and endemic nectarivorous animals.

*Germination and natural regeneration*

None of the seeds from any of the germination experiments germinated. This could be due to several reasons. The Petri dishes or the cotton wool could have been contaminated, but the dishes were rinsed in alcohol prior to the experiments, and the cotton wool was new from an unopened package. Under the given circumstances of working in a field camp, we provided the best possible germination conditions. If the seeds had not been attacked by fungus, they may have germinated after a longer period of time, or perhaps the seeds need to pass through the gut of a specific endemic animal, now locally or globally extinct, other that *P. cepediana* in order to germinate. Alternatively, the seeds might need a special microhabitat, or they may need certain mycorrhizal fungi present before they can germinate. Directed dispersal (Howe & Smallwood 1982) to certain microhabitats suitable for germination and growth may be important for *R. simplex*. Lizards in particular have been suggested and shown to deposit seeds in protected microhabitats such as small cracks and crevices, with positive effects on seed germination and seedling growth (Whitaker 1987; Valido & Nogales 1994; Valido 1999; Wotton 2002). *Phelsuma* geckos often hide in narrow leaf-axils of *Pandanus* or palm species, or in crevices and holes in native trees. The semi-epiphytic *R. simplex* may well require a specific microhabitat created by native vegetation to germinate.

*Biogeography and evolution*

While the monophyly of Rousseaceae s.l. has received strong support (Lundberg 2001; Lundberg & Bremer 2003), the biogeography of the involved genera is still a mystery.

With its basal position in Asterales, it is clear that the lineage of which *R. simplex* is the only extant member is not a neoendemic in Mauritius. It is possible to invoke a relatively recent extreme long-distance dispersal event of *R. simplex* or its ancestral form from at least Papua New Guinea to Mauritius, with subsequent extinction of the lineage from there. However, it is more parsimonious to assume a relatively recent dispersal of *R. simplex* or its ancestral form from a point much closer to Mauritius; e.g. India or Madagascar, with subsequent extinction of the lineage from there. Rousseaceae s.l. could well have evolved while these regions were geographically much closer to Papua New Guinea and Australia than they are today (Kearey & Vine 1996). Indeed, phylogenetic dating has suggested a Cretaceous east Gondwanan (Australia, New Guinea and New Zealand) origin of the Asterales some 100 my ago (Bremer & Gustafsson 1997; Bremer *et al.* 2004).

Little is known about the pollination biology of the three most closely related genera in Carpodetaceae (*Carpodetus*, *Cuttsia* and *Abrophyllum*), but their flowers are all quite small and open. Several thrips (Thysanoptera) species have been observed visiting the flowers of *Carpodetus serratus* (Norton 1984), and *Cuttsia viburnea* is visited by thrips, flies (Diptera) and beetles (Coleoptera) (Williams & Adam 1994; Williams *et al.* 2001). The only known seed disperser of any of the species is the cassowary *Casuarius casuarius*, which eats the black berries of *Abrophyllum ornans* (Crome 1975). Lizard pollination and potential lizard seed dispersal could have evolved *in situ* in Mauritius or earlier in its evolutionary history; e.g. in Madagascar or on some of the now disappeared islands that existed during the last 50 my, between India and where the Mascarene Islands now lie. Some of these once large volcanic islands remain as sunken atolls just below the ocean surface (e.g. the Saya de Malha Bank), and would have been emergent islands with forests during most of the glacial periods of the last 2–3 my (Kearey & Vine 1996; Cheke & Hume in press).

While local dispersal agents of *R. simplex* seeds today may be limited to small lizards, these are not likely to have been the original vector responsible for the arrival of the lineage on Mauritius, because of their relatively rapid gut passage times. It is more likely that the lineage arrived either in the gut of giant tortoises, which have much longer gut passage times (Chapter 5), or in fast, volant animals such as birds or fruitbats. An example of the latter is given by Shilton *et al.* (1999), who found that fruitbats could retain small *Ficus* seeds of a size similar to *R. simplex* seeds (~1 mm) in the gut for up to 12 hours – much longer than normally assumed for fruitbats, and certainly long enough for

inter-island long-distance dispersal. However, based on molecular data, an extreme long-distance dispersal event from Australia to Mauritius has recently been proposed to account for the arrival of *Leiolopisma* skinks in the Mascarene Islands (Austin & Arnold 2006). Hence, lizard-mediated arrival of *R. simplex* in Mauritius cannot be ruled out, even though it is unlikely to have been entirely endozoochorous, given the time span necessary for ocean travel from Australia to Mauritius.

*Conservation management*

During our surveys, we found a total of around 80 adult plants of *R. simplex* (Table 1. It is very likely that further surveys of surrounding areas in Bassin Blanc, Pigeon Wood and Piton Savanne will reveal further individuals. It is almost certain that the population on Le Pouce contains additional plants on the steep northern slopes that we could not survey. However, even if additional plants are located, the known populations would still remain very small and widely scattered, and genetical exchange between populations is unlikely to occur at the moment. We did not find plants in one former location (Kanaka Crater), and the population at Bassin Blanc, described as being 'full of *R. simplex* plants everywhere' in the 1980s (M. Allet, pers. comm.) now contains fewer than 20 scattered individuals. Currently, only the three plants in Pétrin are growing in a weeded and fenced CMA. The Pouce population grows in a Forestry Service nature reserve – which in reality is a small patch of 1–2 ha of native forest that is cut through the middle by a path that every day leads a large number of local and foreign tourists to the summit of Pouce just above the patch. All the other populations are growing in heavily invaded and degraded areas, with only the plants in Pigeon Wood growing within the boundaries of the Black River Gorges National Park. We did not find small, juvenile plants or seedling in any of the populations. Therefore, *R. simplex* must be considered critically endangered and still declining.

Our results show that a reasonably efficient within-population pollination service is being provided by *P. cepediana* geckos – at least in the southern *R. simplex* populations where this gecko co-occurs (Vinson 1976). In the northern population at Pouce, another endemic gecko, *P. ornata*, may be an efficient pollinator. This gecko is known to pollinate other plant species in Mauritius (Nyhagen *et al.* 2001; Olesen *et al.* 2002), and a recent study showed it to be particularly attracted to artificial flowers with yellow and white petals (Beer 2005). The potential role of seed-dispersing lizards in the reproductive ecology of *R. simplex* is less clear. Gut passage through frugivorous lizards can have both

positive (Figueira *et al.* 1994; Valido & Nogales 1994) and negative or neutral (Iverson 1985; Valido & Nogales 1994) effects on seed germination. With our study we were unable to address effects of lizard gut-passage on *R. simplex* seeds, as none of the seeds from any of the treatments germinated. Overall, due to their often high abundances in many insular ecosystems (Rodda & Dean-Bradley 2002), combined with a very generalised diet (Olesen & Valido 2003), lizards could be important pollinators and dispersers for many endangered endemic island plants. A good example of this comes from the Balearic Islands, where Castilla (1999; 2000) showed that the endangered plant *Withania frutescens* (Solanaceae) is dispersed by the lizard *Podarcis lilfordi*.

For *R. simplex*, apart from the previous massive loss of native habitat between the 17th century and the 1980s, one likely continuous cause of rarity and decline is competitive exclusion from the preferred subcanopy strata between 3–6 m in the wet upland forests, where especially strawberry guava *Psidium cattleianum* crowns now form dense canopies, excluding native species. One conservation management option is therefore weeding of the invasive plant species – although at Pouce, this approach may cause the demise of one of the last strongholds of endemic ant species, and open the habitat for incursions by invasive ants (Ward 1990; Lach & Suarez 2005; Fisher 2005). In Chapter 7 we showed that invasive *Technomyrmex albipes* ants have a detrimental impact on the pollination and seed dispersal interactions between *R. simplex* and *P. cepediana*. Hence, conservation management of the last remaining *R. simplex* populations must take these findings into account. Moreover, in the short term, weeding of invasive species will reduce structural habitat diversity, which leads to lower densities of *Phelsuma* geckos (Padayatchy 1998; Harmon 2005) until native vegetation has regrown. Therefore, gradual weeding, leaving patches of dense invasive vegetation for a number of years, may be better than removing all invasive vegetation at once, as is currently practised in habitat restoration efforts in Mauritius. The only way the National Parks and Conservation Service and the Forestry Service have been able to propagate *R. simplex* is with cuttings from adult plants, but these are hard to get to grow and survive in nurseries (R. Rutty, pers. comm.). Therefore, further study on how the seeds can be brought to germinate either *in situ* or *ex situ* is urgently required.

## ACKNOWLEDGEMENTS

Thank you to Mario Allet, Raj Rutty, Vincent Florens and Jean-Claude Sevathian for sharing their knowledge about several of the *Roussea simplex* populations; to the National Parks and Conservation Service and the Forestry Service for permission to work in the forests and for general assistance, and to the staff and volunteers of the Mauritian Wildlife Foundation for neverending support and friendship. Also thank you to Johannes Lundberg and Mats Gustafsson for sharing unpublished manuscripts. The project was funded by the Swiss National Science Foundation (grant no. 631-065950 to CBM)

## REFERENCES

ARAGON, P., LOPEZ, P. & MARTIN, J. (2006) Roles of male residence and relative size in the social behavior of Iberian rock lizards, *Lacerta monticola*. *Behavioral Ecology and Sociobiology* 59: 762-769.

AUSTIN, J.J. & ARNOLD, E.N. (2006) Using ancient and recent DNA to explore relationships of extinct and endangered *Leiolopisma* skinks (Reptilia : Scincidae) in the Mascarene islands. *Molecular Phylogenetics and Evolution* 39: 503-511.

BAWA, K.S. (1990) Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399-422.

BEER, K. (2005) Preference for floral traits by endemic daygeckos (*Phelsuma ornata*) on Mauritius (M.Sc.). Zurich: University of Zurich.

BREMER, K. & GUSTAFSSON, M.H.G. (1997) East Gondwana ancestry of the sunflower alliance of families. *PNAS* 94: 9188-9190.

CASTILLA, A.M. (1999) *Podarcis lilfordi* from the Balearic islands as a potential disperser of the rare Mediterranean plant *Withania frutescens*. *Acta Oecologica* 20: 103-107.

CASTILLA, A.M. (2000) Does passage time through the Lizard *Podarcis lilfordi*'s guts affect germination performance in the plant *Withania frutescens*? *Acta Oecologia* 211: 119-124.

CHEKE, A.S. & HUME, J.P. (in press) *Lost land of the Dodo*. London, UK: Christopher Helm.

CONNELL, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: *Dynamics of populations* (Boer P.J.D., Gradwell G.R., eds), pp. 298-312. Centre for Agricultural Publication and Documentation, Wageningen.

CROME, F.H.J. (1975) Some observations on the biology of the Cassowary in Northern Queensland. *Emu* 76: 8-14.

FIGUEIRA, J.E.C., VASCONCELLOSNETO, J., GARCIA, M.A. & DESOUZA, A.L.T. (1994) Saurocory in *Melocactus violaceus* (Cactaceae). *Biotropica* 26: 295-301.

FISHER, B.L. (2005) A new species of *Discothyrea* Roger from Mauritius and a new species of *Proceratium* Roger from Madagascar (Hymenoptera: Formicidae). *Proceedings of the California Academy of Sciences* 56: 657-667.

FRIEDMANN, F. (1988) *Fleurs rares des Iles Mascareignes*. Port Louis: L'Ile aux Images Editions.

GODÍNEZ-ÁLVAREZ, H. (2004) Pollination and seed dispersal by lizards: a review. *Revista Chilena de Historia Natural* 77: 569-577.

GUSTAFSSON, M.H.G. & BREMER, K. (1997) The circumscription and systematic position of Carpodetaceae. *Australian Systematic Botany* 10: 855-862.

GUSTAFSSON, M.H.G. (in press) Carpodetaceae. In: *The families and genera of vascular plants. Vol. 8: Asterales* (Kadereit, J.W., Jeffrey, C., eds). Berlin: Springer-Verlag.

HANSEN, D.M. (2005) Pollination of the enigmatic Mauritian endemic *Roussea simplex* (Rousseaceae): Birds or geckos? *Ecotropica* 11: 69-72.

HARMON, L.J. (2005) Competition and community structure in day geckos (*Phelsuma*) in the Indian Ocean. Saint Louis, Missouri: Washington University.

HOWE, H.F. & SMALLWOOD, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201-228.

IVERSON, J.B. (1985) Lizards as seed dispersers. *Journal of Herpetology* 19: 292-293.

JANZEN, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist* 104: 501-529.

KEAREY, P. & VINE, F.J. (1996) *Global tectonics*. Oxford: Blackwell.

KELLY, D., LADLEY, J.J. & ROBERTSON, A.W. (2004) Is dispersal easier than pollination? Two tests in new Zealand Loranthaceae. *New Zealand Journal of Botany* 42: 89-103.

KOONTZ, J.A., LUNDBERG, J. & SOLTIS, D.E. (in press) Rousseaceae. In: *The families and genera of vascular plants. Vol. 8: Asterales* (Kadereit, J.W., Jeffrey, C., eds). Berlin: Springer-Verlag.

LACH, L. & SUAREZ, A.V. (2005) One restoration effort does not beget another: native ants in Mauritius do not benefit from plant restoration efforts. *Abstract – XIX Annual Meeting of the Society for Conservation Biology*. Brasilia.

LAWTON, J. (1995) Ecology of the afterlife. *Oikos* 73: 145-147.

LOPEZ, P. & MARTIN, J. (2001) Fighting rules and rival recognition reduce costs of aggression in male lizards, *Podarcis hispanica*. *Behavioral Ecology and Sociobiology* 49: 111-116.

LUNDBERG, J. (2001) The asteralean affinity of the Mauritian *Roussea* (Roussaceae). *Botanical Journal of the Linnean Society* 137: 267-276.

LUNDBERG, J. & BREMER, K. (2003) A phylogenetic study of the order Asterales using one morphological and three molecular data sets. *International Journal of Plant Sciences* 164: 553-578.

MAUREMOOTOO, J.R., CHEKE, A.S. & WATT, I. (in press) Mauritius & Rodrigues historical context. In: *State of the Hotpots: Madagascar & Indian Ocean Islands* (Edmond R., Langrand O.L., Galindo-Leal C., eds). Washington D.C.: Conservation International.

NICOLL, M.A.C., JONES, C.G. & NORRIS, K. (2003) Declining survival rates in a reintroduced population of the Mauritius kestrel: evidence for non-linear density dependence and environmental stochasticity. *Journal of Animal Ecology* 72: 917-926.

NORTON, S.A. (1984) Thrips pollination in the lowland forests of New Zealand. *New Zealand Journal of Ecology* 7: 157-164.

NYHAGEN, D.F., KRAGELUND, C., OLESEN, J.M. & JONES, C.G. (2001) Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. *Journal of Tropical Ecology* 17: 755-761.

OLESEN, J.M., ESKILDSEN, L.I. & VENKATASAMY, S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distribution* 8: 181-192.

OLESEN, J.M. & VALIDO, A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution* 18: 177-181.

PADAYATCHY, N. (1998) The short term effects of weeding on populations of endemic day geckos in a Mauritian upland forest conservation management area (B.Sc. (Hons.)). Port Louis: University of Mauritius.

PERNETTA, A.P., BELL, D.J. & JONES, C.G. (2005) Macro- and microhabitat use of Telfair's skink (*Leiolopisma telfairii*) on Round Island, Mauritius: implications for their translocation. *Acta Oecologica* 28: 313-323.

R DEVELOPMENT CORE TEAM (2006) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

RODDA, G.H. & DEAN-BRADLEY, K. (2002) Excess density compensation of island herpetofaunal assemblages. *Journal of Biogeography* 29: 623-632.

SAZIMA, M., BUZATO, S. & SAZIMA, I. (2003) *Dyssochroma vitidiflorum* (Solanaceae): a reproductively bat-dependent epiphyte from the Atlantic rainforest in Brazil. *Annals of Botany* 92: 725-730.

SCHWENK, K. (1993) Are geckos olfactory specialists? *Journal of Zoology* 229: 289-302.

SCOTT, A.J. (1997) Brexicacées. In: *Flore des Mascareignes* (Bosser T.C.J., Guého J., Marais W., eds). Reduit: The Mauritius Sugar Industry Research Institute; 1-3.

SHILTON, L.A., ALTRINGHAM, J.D., COMPTON, S.G. & WHITTAKER, R.J. (1999) Old World fruit bats can be long-distance seed dispersers through extended retention of viable seeds in the gut. *Proceedings of the Royal Society of London Series B-Biological Sciences* 266: 219-223.

SORIANO, P.J. & RUIZ, A. (2002) The role of bats and birds in the reproduction of columnar cacti in the northern Andes. In: *Columnar cacti and their mutualists* (Fleming, T.H., Valiente-Banuet, A., eds). Tucson: University of Arizona Press; 241-263.

TANIGUCHI, Y., HISATOMI, O., YOSHIDA, M. & TOKUNAGA, F. (1999) Evolution of visual pigments in geckos. *FEBS Letters* 445: 36-40.

TRAVESET, A. & SAEZ, E. (1997) Pollination of Euphorbia dendroides by lizards and insects: Spatio-temporal variation in patterns of flower visitation. *Oecologia* 111: 241-248.

VALIDO, A. (1999) Ecología de la dispersión de semillas por los lagartos endémicos canarios (g. *Gallotia*, Lacertidae) (Ph.D. Thesis). Tenerife, Canary Islands: University of La Laguna.

VALIDO, A. & NOGALES, M. (1994) Frugivory and seed dispersal by the lizard *Gallotia galloti* (Lacertidae) in a xeric habitat of the Canary Islands. *Oikos* 70: 403-411.

VALIDO, A. & OLESEN, J.M. (In press) Importance of lizards as frugivores and seed dispersers. In: *Seed dispersal: theory and its application in a changing world* (Dennis A., Green R., Schupp E., Westcott D., eds): CABI Publishing.

VAUGHAN, R.E. & WIEHE, P.O. (1937) Studies on the Vegetation of Mauritius: I. A Preliminary Survey of the Plant Communities. *Journal of Ecology* 25: 289-343.

VINSON, J. & VINSON, J.-M. (1969) The saurian fauna of the Mascarene Islands. *Mauritius Institute Bulletin* 6: 203-320.

VINSON, J.M. (1976) The saurian fauna of the Mascarene Islands. II. The distribution of *Phelsuma* species in Mauritius. *Bulletin of the Mauritius Institute* 8: 177-195.

WHITAKER, A.H. (1987) The roles of lizards in New Zealand plant reproductive strategies. *New Zealand Journal of Botany* 25: 315-328.

WILLIAMS, G.A., ADAM, P. & MOUND, L.A. (2001) Thrips (Thysanoptera) pollination in Australian subtropical rainforests, with particular reference to pollination of Wilkiea huegeliana (Monimiaceae). *Journal of Natural History* 35: 1-21.

WOTTON, D.M. (2002) Effectiveness of the common gecko (*Hoplodactylus maculatus*) as a seed disperser on Mana Island, New Zealand. *New Zealand Journal of Botany* 40: 639-647.

# CHAPTER 7

**The invasive ant *Technomyrmex albipes* disrupts gecko pollination and seed dispersal of the critically endangered endemic Mauritian plant *Roussea simplex***

"What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions".

– Janzen, 1974

**ABSTRACT**

In Mauritius, the endemic blue-tailed day-gecko *Phelsuma cepediana* is currently the sole pollinator and seed disperser of the critically endangered endemic plant *Roussea simplex* (Rousseaceae). The flowers and fruits on this plant are often infested with the invasive ant *Technomyrmex albipes*, which forages on the nectar and fruit pulp, and tends honeydew-producing mealybugs on the fruits. Here, we experimentally explore how the presence of this ant influences the geckos foraging at flowers and fruits of *R. simplex* by removing and excluding ants from infested flowers and fruits. Gecko visitation rates to uninfested control flowers and fruits, and flowers and fruits where ants had been removed and excluded, were much higher than to infested flowers and fruits. Resulting seed set in ant-infested flowers was greatly reduced, compared to uninfested control flowers. Similarly, on ant-infested fruits, very few seeds were likely to be ingested by the geckos. Thus, *T. albipes* monopolises the use of flowers and fruit, and displaces the geckos by aggressive interference competition, disrupting both the pollination and the seed dispersal interactions of *R. simplex*. For a critically endangered plant like *R. simplex*, this is of urgent conservation concern.

## INTRODUCTION

Pollination and seed dispersal are two key processes in the reproductive ecology of most plants, and in the tropics they are often mediated by animals (Howe & Smallwood 1982; Bawa 1990). Recent reviews have highlighted that on islands, these two ecosystem functions are often provided by lizards (Olesen & Valido 2003; Godínez-Álvarez 2004; Valido & Olesen in press). A good example of this, combining both lizard pollination and lizard seed dispersal, is found on the island of Mauritius in the Indian Ocean. Here, the endemic blue-tailed day-gecko *Phelsuma cepediana* is currently the sole pollinator and seed disperser of the critically endangered endemic plant *Roussea simplex* (Rousseaceae) (Chapter 6). The geckos feed on nectar at the flowers and on pulp with embedded tiny seeds at the fruits. The large yellow flowers present plentiful standing crops of nectar for 6–8 days, while fruits present fresh pulp over a period of 4–7 days. *Roussea simplex* flowers are also visited by other animals, but only rarely, and they do not serve as legitimate pollinators (Hansen 2005; Chapter 6). However, one of the other flower-visiting species, the introduced and invasive white-footed ant *Technomyrmex albipes*, can be found in large numbers on and around some flowers and fruits on *R. simplex* plants. While foraging for nectar in the flowers or for sweet liquids in the fruit pulp, or while tending honeydew-producing mealybugs (Hemiptera: Pseudococcidae) on developing fruits, *T. albipes* ants quickly become extremely aggressive when disturbed, and will immediately attack any intruding animal or object (pers. obs.). Furthermore, the ants build constructions of dirt (galleries) around resources they like to protect, be it a large nectar pool or aphids or scale insects that they milk for honeydew. Preliminary observations suggested that this aggressive behaviour could deter or hinder the pollinating and seed dispersing *P. cepediana* geckos, thereby negatively impacting the reproductive success of *R. simplex*.

The impacts of six of the most invasive ant species on native ecosystems have recently been reviewed (Holway *et al.* 2002; Ness & Bronstein 2004). Both reviews mention the status of *T. albipes* as a 'candidate' invasive species, but stress that too little is known about its ecology in invaded areas to assess its impact on native ecosystems. Despite growing concern about detrimental effects caused by invasive ants (Holway *et al.* 2002; Lach 2003; Ness & Bronstein 2004), only few studies have investigated the effect of invasive ants on mutualistic pollination and seed dispersal interactions, and more research is urgently needed (Traveset & Richardson 2006). Recently, a few studies have shown that invasive ants can negatively affect the pollination interactions between native plants and

flower-visiting arthropods (Blancafort & Gomez 2005; Lach 2005). Similarly, invasive ants have been shown to disrupt or negatively affect native ant-mediated seed dispersal interactions (Bond & Slingsby 1984; Christian 2001; Zettler *et al.* 2001; Carney *et al.* 2003; Gomez & Oliveras 2003; Ness 2003; Ness 2004; Ness *et al.* 2004; Oliveras *et al.* 2005). However, despite the well-known aggressiveness of many invasive ants (Holway *et al.* 2002) and their detrimental effects on vertebrates (e.g. Feare 1999; Meek 2000; Jourdan *et al.* 2001), no studies have investigated the potential disruption of vertebrate-mediated pollination and seed dispersal mutualisms by invasive ants. Lach (2003; 2005) suggested that pollination interactions between endemic nectarivorous Hawaiian birds and the plants they pollinate may be susceptible to exploitative or interference competition by invasive ants, but sadly this study provided no evidence to support this idea.

In this study, we experimentally address the specific questions: 1) Does the presence of *T. albipes* ants on flowers and fruits of *R. simplex* affect the behaviour of the pollinating and seed dispersing gecko *P. cepediana*? 2) If so, does this affect the reproductive success of *R. simplex*? We discuss the implications of our findings for the conservation management of *R. simplex* and other endangered plants in Mauritius.

## MATERIALS AND METHODS

*Study site and study species*

In November 2004 – February 2005, we studied the effect of the presence of *T. albipes* ants on *P. cepediana* geckos foraging at *R. simplex* flowers and fruits in two *R. simplex* populations, Pétrin and Bassin Blanc. *Roussea simplex* is a critically endangered endemic plant, with around 85–90 known individuals in seven populations. Detailed descriptions of the study sites, *R. simplex*, and *P. cepediana* are given in Chapter 6. Pétrin is a 6.2 ha fenced and weeded conservation management area (CMA) within the Black River Gorges National Park, and three large *R. simplex* plants are found within the CMA. The Bassin Blanc *R. simplex* population consists of 17 adult plants within an area of ~ 0.5 ha on a steep slope heavily degraded by alien plants with only few native trees left.

*Technomyrmex albipes* is native to Malaysia and Indonesia, but has spread to many parts of the tropics and subtropics during the last few hundred years. It was first reported from the neighbouring island of La Réunion in 1895, and from Mauritius in 1946 (Donisthorpe 1946), but it is likely to have been in Mauritius for longer. By 1990 it was considered a serious invader of native habitats in Mauritius (Ward 1990). In several regions, including North America, ant species looking superficially similar to *T. albipes*

have been reported as invasive *T. albipes* – but they are actually the closely related species *T. difficile*, *T. vitiensis* or *T. pallipes* (B. Bolton, pers. comm.). Hence, even though the ecology, and thus the impacts, of the different species is likely to be similar, caution is advised when investigating invasive *Technomyrmex* spp., and ant taxonomists should be consulted in cases of doubt. Part of *T. albipes*' success as an invasive tramp species is due to its unusual colony structure, which is geared for producing high numbers of offspring: *Technomyrmex albipes* is a polygynous (multiple queens) and polydomous (multiple colonies) species with an intercaste of wingless females, which can produce trophic eggs that are fed to the larvae (Tsuji & Yamauchi 1994). Furthermore, polydomous ants are not restricted to central-place foraging, but can quickly utilise new resources by making small or large nests next to new resources (Holway & Case 2000). *Technomyrmex albipes* is very attracted to sweet sugar solutions, and often forages on nectar or honeydew produced by aphids, scale insects or mealybugs (Koptur & Truong 1998; Heenan 1999; Warner & Scheffrahn 2005). In fact, utilisation and monopolisation of liquid carbohydrate resources may be one of the reasons for the high abundance and activity level of invasive ants (Holway *et al.* 2002). One study demonstrated that *T. albipes* has a preference for fructose sugar (Koptur & Truong 1998), while another study showed that hexose sugars and sucrose were equally preferred (Warner & Scheffrahn 2005). *Roussea simplex* nectar is composed of hexose sugars (53% glucose and 47% fructose), is of a relatively low sugar concentration (6.5–14%), and is produced in large quantities of up to several hundred μL per flower per day (Chapter 6).

In the *R. simplex* flowering seasons in 1998–1999, 1999–2000, and 2003–2004, *T. albipes* was often observed to build thin, delicate structures of debris and insect silk (galleries) across the entire corolla of several flowers on each *R. simplex* plant (Fig. 1A,C), repairing and maintaining them as anthers wilted and fell off. One small hole, typically at the edge where two petals fused, was left open and usually guarded by one or two ants (Fig. 1B). For unknown reasons, in our study season in 2004–2005 the ants did not build galleries across flowers until very late in the flowering season, and then only at very few flowers. We were therefore unable to experimentally investigate the effect of these structures on gecko foraging. However, throughout the flowering season, *T. albipes* still foraged for nectar in large numbers (5–30 ants at any one time) at flowers of most *R. simplex* plants. While ants were sometimes observed walking across the stigma of a *R. simplex* flower, the ants were never observed with any of the large, pale yellow pollen grains adhering to their bodies, and were therefore not providing any pollination service to

*R. simplex*. In fact, the few times we observed ants walking across anthers, they got stuck on the slimy pollen substance (Fig. 1I). Furthermore, on developing *R. simplex* fruits, *T. albipes* often tended mealybugs (Hempitera: Pseudococcidae) (Fig. 1H), protected by small structures made of the same material used across flower corollas (Fig. 1G). When the fruits were ripe and opened to present a slimy pulp with embedded seeds, *T. albipes* ants would also forage on the sweet liquid of the pulp (Fig. 1F, G).

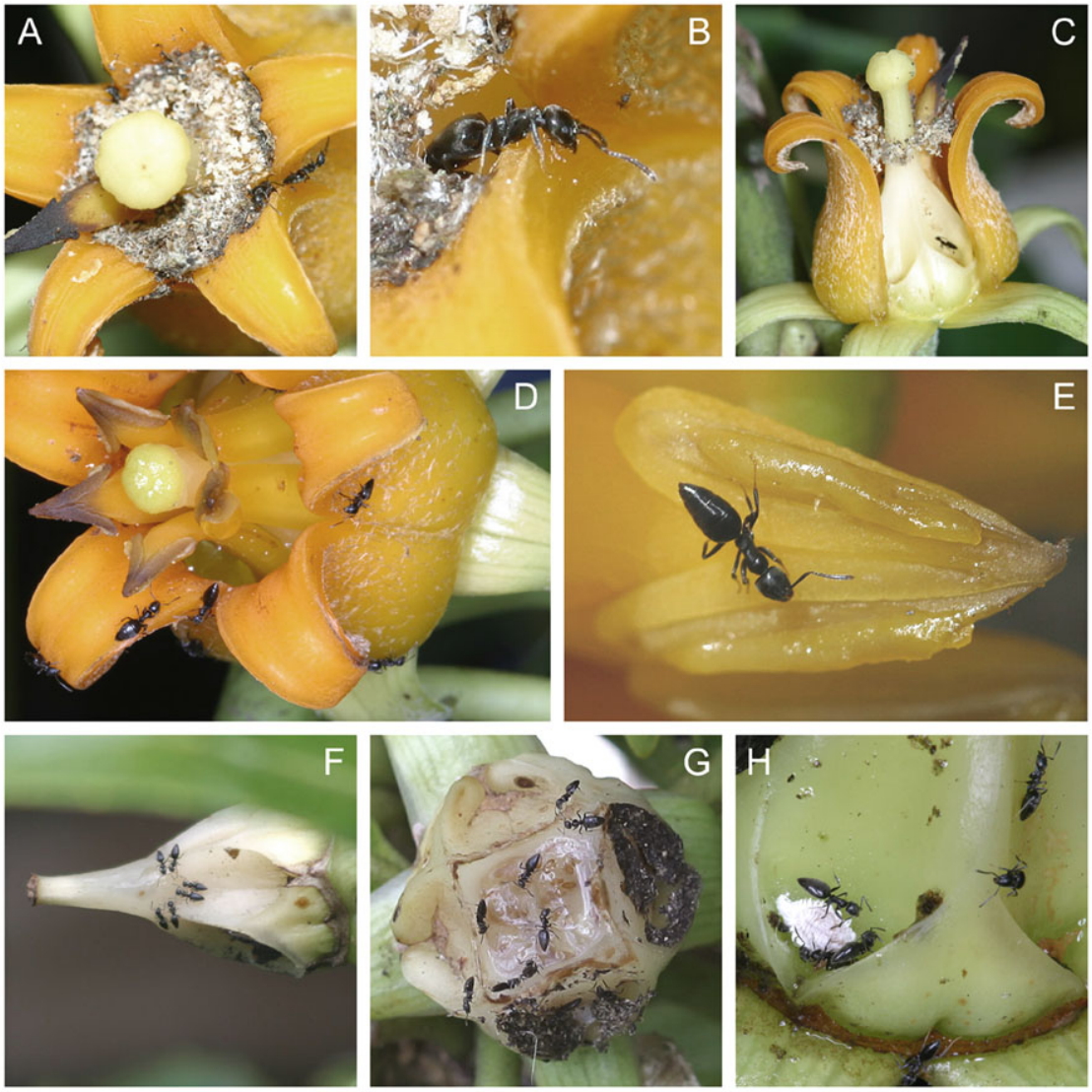



**FIGURE 1.** Infestation by *Technomyrmex albipes* ants on flowers and fruits of *Roussea simplex*. (A) The delicate dirt gallery, built across the corolla opening of a flower. (B) The single opening, guarded by 1-2 ants. (C) One petal removed, showing a cross-section of a flower with the dirt gallery across the top. (D) An open flower with a group of ants on guard and nectar foraging. (E) An ant stuck to the slimy pollen substance of an anther. (F) Ants foraging at the sweet liquid exudated from an almost ripe fruit, ready to open (cf. Chapter 6, Fig. 2G). (G) Ants foraging at fruit pulp, and tending mealybugs in the two small galleries built on the sides of the fruit. (H) Gallery removed to show ants tending a mealybug on a fruit.

*Ant exclusion experiments*

We used short-term ant-exclusion experiments to investigate the effects of ants on the foraging behaviour of *P. cepediana* geckos at *R. simplex* flowers and fruits. Ideally, we would have excluded ants or geckos, respectively, throughout anthesis and fruiting to investigate effects on seed set and pulp removal rates. However, *R. simplex* has a dense, almost divaricate, growth form, with brittle branches and leaves that snap easily when bent. Excluding geckos from flowers or fruits requires covering 25–30 cm of a branch with grease and securing a minimum distance of 40–50 cm to neighbouring vegetation (Chapter 3). Furthermore, *T. albipes* ants are very adept at using overlapping branches or leaves to reach favoured food sources (Warner 2003). Consequently, it was not possible to exclude geckos or ants from flowers or fruits for the required minimum of 8–10 days without seriously injuring or altering the plants.

Short-term ant exclusion experiments were set up on days with sunshine or sunny/cloudy weather with no rain, in the flowering and fruiting seasons of *R. simplex* in south-western Mauritius in November–December 2004 and January–February 2005, respectively. Ant-exclusion experiments at flowers and fruits were done at two *R. simplex* populations, Pétrin (3 plants in population, all used for experiments) and Bassin Blanc (17 plants in population, four used for experiments; overlap of two plants between experiments with flowers and fruits). Ants were excluded by wrapping 2–4 cm of brown tape around a branch between 30 and 40 cm long and with 1–3 open flowers or 1–3 ripe fruits, and covering the tape with a thin layer of silicon-based car grease. All *T. albipes* ants on the excluded branches and flowers were gently removed by blowing either directly or through a 0.5 cm diameter drinking straw, and by using a small paintbrush, while ensuring no spillage of nectar from the flowers or pulp from the fruits. After all ants had been removed, any branches or leaves touching the excluded branches were gently bent 2–5 cm away from the excluded branch, and secured to surrounding branches or leaves with wooden clothes pegs and/or string. Thus, geckos were free to access all branches and flowers, while ants could not move onto the branches excluded with grease. As controls, we observed a similar number of a) flowers or fruits that still had ants foraging, and b) flowers or fruits that did not have ants foraging. Immediately after the exclusion experiments, the greased tape was removed and branches were returned to their original positions. We waited a minimum of 10 days between repeating the experiment at any one individual plant.

Gecko visitation observations were made with Leica 10 × 32 mm binoculars from a distance of 4–5 metres, with the observer either mostly covered by vegetation or by a 1 × 3 m lightweight camouflage net draped over the head and shoulders. After setting up the observation post, the observer remained as motionless as possible for 20 minutes before starting to record gecko visitation, to allow nearby animals to get used to the presence of a human. We recorded number of gecko visits per fruit or flower per hour, and the duration of each visit in seconds. Observation periods for the ant exclusion experiments were a subset of observation periods used in Chapter 6; thus the flowers we use here for the control category without ants have already been presented as part of the data in Chapter 6. Observation periods for flowers were 60 or 90 min, while those for fruits were 90 or 120 min.

*Levels of ant infestation*

Typically, not all flowers or fruits on any one *R. simplex* plant are infested with foraging ants, and not all flowers or fruits have the small dirt galleries built on them. We therefore surveyed levels of ant infestation at plant level, and at the level of flowers and ripe, open fruits. This was done at both study populations and the populations at Piton Savanne (23 plants), and Grand Bassin (for fruiting plants only; 7 plants; see Chapter 6). We recorded the number of plants that had *T. albipes* ants on them and, on each of these plants, surveyed 20 randomly chosen flowers or ripe or nearly ripe fruits for presence of a) > 5 ants foraging for nectar (flowers), or b) galleries containing ants and mealybugs (fruits).

*Seed set*

While ants in 2004–2005 did not, as often as previously observed, build small galleries across the corolla opening of *R. simplex* flowers, they did forage more or less constantly at the same flowers throughout anthesis (pers. obs.). Depending on the time during anthesis scouting ants selected a flower, the male reproductive success of a flower (i.e. pollen removal) may have been less affected than the female reproductive success (i.e. seed set). We quantified the impact on female reproductive success only. During November 2004, we marked three ant-infested male phase flowers on each of the three plants in Pétrin, and three ant-infested male phase flowers on three plants at Bassin Blanc. We selected male phase ant-infested flowers, as ants were likely to be present at the flowers throughout the female phase as well. In late February 2005 the resulting developing fruits were harvested and number of seeds was recorded. Seeds were counted by cutting ~2 mm cross-sections

off the developing fruits, and counting the seeds in groups of five or ten seeds with a dissecting needle under a Nikon 20× magnification field dissecting microscope. We compared numbers of seeds in developing fruits from ant-infested flowers to numbers of seeds in developing fruits from non-infested flowers from Pétrin and Bassin Blanc (using the open-pollinated control flowers from the pollination experiments in Chapter 6; N = 18 flowers).

*Statistical analyses*

All statistical analyses were done with R.2.3.1 (R Development Core Team 2006). Differences in visitation rates were analysed with Kruskal-Wallis tests. Differences in ant infestation levels were investigated with a generalised linear model with a quasibinomial error structure to account for overdispersed data. Seed set of ant-infested and open-pollinated control flowers were compared with a Wilcoxon-Mann-Whitney test.

## RESULTS

*Ant exclusion experiments*

There was a very strong negative effect of the presence of ants on gecko foraging at both flowers and fruits. Gecko visitation rates at ant-infested flowers and fruits were zero or near-zero in both *R. simplex* study populations, whereas flowers and fruits, where ants had been removed and excluded, achieved visitation rates similar to those of control flowers and fruits without ants (Fig. 2A,B; Kruskal-Wallis tests: all P-values < 0.001). Geckos often remained at a distance of up to 1 m and watched a cluster of fruits or flowers for between one to 15–20 minutes before making the decision to forage. On the few occasions, where geckos attempted to forage at ant-infested flowers and fruits, the mean duration of visits was with 2–7 seconds very short, whereas geckos foraged at ant-excluded and control flowers and fruits for much longer (Fig. 2C,D). When attempting to forage at ant-infested flowers and fruits, geckos quickly reacted negatively by retracting and shaking their heads and/or feet, which were the main targets of the attacking ants. If there were only 1–3 patrolling ants on a flower, the geckos often stayed nearby and watched them, only entering the flower after the ants had left. However, if there was a regular activity of ants going to and from a flower (meaning that anywhere from 5 to 30 ants could be found on and inside the flower), no geckos approached the flower.

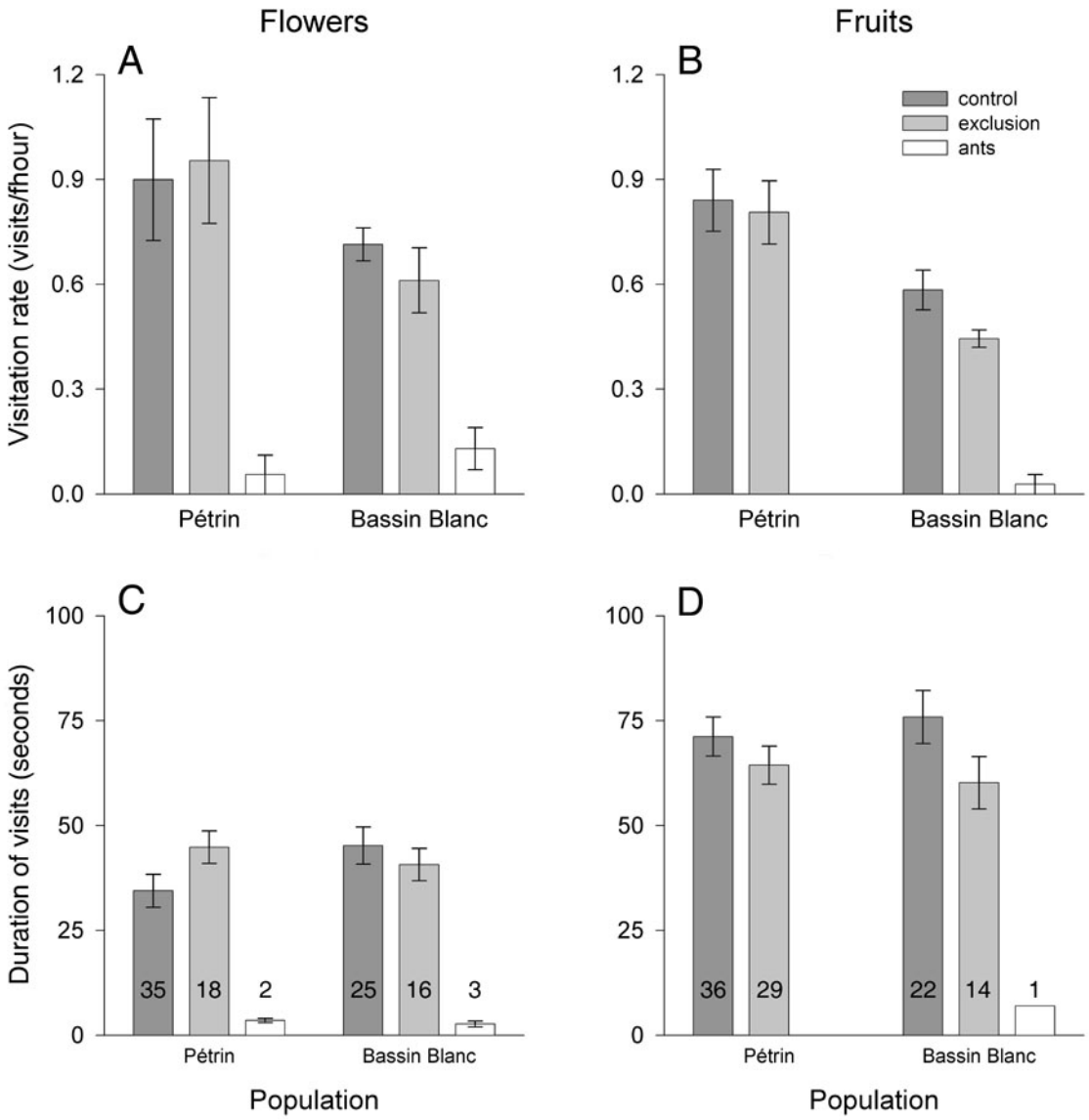



**FIGURE 2.** Visitation rates (A, B) and duration of visits (C, D) of *Phelsuma cepediana* geckos at flowers and fruits of *Roussea simplex* in the two study populations, Pétrin and Bassin Blanc. 'Control' flowers and fruits did not have ants present, 'exclusion' flowers and fruits had ants present that were removed during experimental periods, and 'ants' flowers and fruits had ants present prior to and during experimental periods. Bars are mean values across flowers/fruits observed ± 1SE. Sample size of each bar in (A) and (B) = six observation periods. Sample sizes of bars in (C) and (D) are shown for each bar.

*Levels of ant infestation*

The majority of flowering and fruiting *R. simplex* plants in all surveyed populations were infested with *T. albipes* (67–100%; Table 1). On some plants, the ants had even built small nests containing several hundred workers, eggs and larvae in rotting, hollow *R. simplex* branches. Levels of ant infestation were higher on fruits (43.3 ± 2.9%, N = 28 plants) than in flowers (32.1 ± 3.1%, N = 44 plants; $F_{1, 70} = 6.42$, P = 0.014). There was no difference in ant infestation levels between populations ($F_{3, 67} = 0.81$, P = 0.49), nor was there any interaction between ant infestation levels and population ($F_{2, 65} = 1.66$, P = 0.20).

**TABLE 1.** Infestation rates of *Techomyrmex albipes* ants on *Roussea simplex* plants, flowers, and fruits.

| Population | # plants flowering and fruiting | # flowering plants infested | Flowers infested per plant | # fruiting plants infested | Fruits infested per plant |
|---|---|---|---|---|---|
| Pétrin | 3 | 3 (100%) | 30.0 ± 13.2% | 3 (100%) | 58.3 ± 7.3% |
| Bassin Blanc | 14 | 11 (79%) | 37.8 ± 4.9% | 13 (76%) | 40.8 ± 11.7% |
| Piton Savanne | 21 | 14 (67%) | 28.2 ± 3.7% | 21 (100%) | 45.5 ± 12.1% |
| Grand Bassin | 7[1] | NA[1] | NA[1] | 7 | 35.0 ± 6.2% |

[1] This population was only surveyed during the fruiting season.

*Seed set*

Significantly more seeds were produced in open-pollinated non-infested flowers (mean ± SD: 505 ± 219 seeds; N = 18) than in ant-infested flowers (87 ± 82 seeds; N = 15, one developing fruit in Pétrin and two at Bassin Blanc were lost for unknown reasons; W = 9.0, P < 0.001). Using the average number of 2572 ovules per *R. simplex* flower (see Chapter 6), the recorded seed numbers correspond to ~20% seed set in open-pollinated non-infested flowers, and ~3% seed set in ant-infested flowers, respectively. There were several developing fruits from ant-infested flowers that did not develop any seeds at all (N = 5 developing fruits), which mirrors the zero seed set results of autogamous flowers in Chapter 6, and indicates that these flowers were not pollinated.

## DISCUSSION

The disruption of mutualisms by invasive species is one of the more insidious threats to native and endemic biodiversity, and few studies have so far attempted to quantify or investigate the issue experimentally (Traveset & Richardson 2006). This is particularly true for studies of invasive ants, which are mostly correlative or based on observations,

rather than having an experimental approach (Holway *et al.* 2002). In our study we used a simple experimental approach to investigate the effects of an invasive ant species on mutualistic interactions in Mauritius. Our results provide the first evidence of a disruption of vertebrate-mediated mutualistic pollination and seed dispersal interactions by invasive ants. We clearly showed that a presence of the invasive ant *Technomyrmex albipes* on flowers and fruits of *Roussea simplex* had a dramatic negative impact on the foraging of *Phelsuma cepediana* geckos at flowers and fruits. Furthermore, there was a large reduction in seed set in fruits developing from ant-infested flowers, compared to non-infested flowers. The greater rate of ant infestation on fruits, compared with that on flowers, was most likely a result of fruits offering a potential resource for ant-tended honeydew-producing mealybugs for several months. Therefore, once a fruit was ripe, the chance that ants were already present, tending mealybugs inside galleries on the fruits, was relatively high. We did not record fruit pulp/seed removal rates at ant-infested fruits versus non-infested fruits, as it was impossible to do so non-invasively, and because the fruits secrete pulp continuously over several days. It is clear, however, from the very low foraging rate at ant-infested fruits, that gecko seed ingestion must be close to zero, especially as geckos usually only ingested seeds after a period of licking had 'pushed' a lump of pulp (with embedded seeds) together, which the geckos would then grab and swallow. In our study season in 2004–2005, *T. albipes* did not build the previously observed galleries across corollas of *R. simplex*. However, observations of such flowers in the flowering season of 2003–2004, and of the few flowers with galleries late in the 2004–2005 season, revealed that geckos never attempted to enter them. It is therefore likely that no pollen would be removed or deposited at such flowers, and that both male and female reproductive success will be zero. Overall, we conclude that the pollination and seed dispersal interactions with *P. cepediana* geckos are disrupted to a large, sometimes almost total, extent in *R. simplex* flowers and fruits that are infested with *T. albipes*. However, while the majority of *R. simplex* plants in all populations were infested with *T. albipes*, current population densities of *T. albipes* are seemingly not high enough to allow the ants to monopolise all flowers or fruits on a plant.

Our study provides evidence that *T. albipes*, at least in Mauritius, should not only be regarded as a 'candidate' invasive ant species, but be considered a seriously invasive species with complex negative interactions with endemic species, on par with the already well-known and well-studied invasive ant species listed in Holway *et al.* (2002) and Ness and Bronstein (2004).

*Interference or exploitative competition?*

In flowers from which we excluded ants, nectar standing crop was always large, typically estimated to be several hundred µL. *Roussea simplex* flowers can produce several hundred µL per day, both during day and night (Chapter 6), and even large numbers of ants per flower were not able to exploit all of it. In fact, the variation in nectar standing crop of flowers with foraging ants may be smaller than in flowers that are visited by geckos, as we often observed low standing crops of nectar in flowers that were visited by geckos (Chapter 6). Therefore, from a purely reward- or resource-oriented point of view, flowers with foraging ants may be *more*, not less attractive to geckos. On ripe fruits, ants primarily tended the mealybugs, but foraged on liquid parts of the pulp as well (Fig. 1G). However, even more so than in flowers, ants were unable to exploit this resource in full. Ant-infested fruits often had large lumps of semi-dry pulp hanging on them, which were never consumed by geckos, but eventually dropped down to the ground (see Chapter 6, Fig. 2I).

These patterns, combined with our results from the ant exclusion experiments and observations of gecko behaviour, strongly suggest that the disruption of the pollination and seed dispersal interactions between *P. cepediana* and *R. simplex* are due to aggressive interference competition by *T. albipes*. Our findings supplement the results of Hansen *et al.* (2002), who found that introduced honey bees competitively excluded endemic nectarivorous birds from two other endemic plants in Pétrin, *Sideroxylon cinereum* and *S. puberulum* (Sapotaceae), by emptying flowers of nectar. However, the flowers of *S. cinereum* and *S. puberulum* are much smaller than those of *R. simplex*, and contain only 4–6 µL of nectar on average – amounts that are easily removed after just a few honey bee visits. Furthermore, no antagonistic interactions were observed between the honey bees and the nectarivorous birds. In general, Hansen *et al.* (2002) argued that island mutualisms may be more susceptible to disruption from exploitative competition with resource-harvesting social hymenopterans (bees and wasps) than comparable mainland interactions – mostly due to an original common lack of these groups on many islands, and the highly generalised nature of many insular species interactions. With our study, we supplement these suggestions by including invasive ants as potential aggressive interference competitor of native and endemic nectar-feeding animals in Mauritius. Additionally, in plant species with less nectar than *R. simplex*, *T. albipes* may also compete exploitatively. In a study of three different nectar-thieving invasive ant species in Hawai'i, Lach (2005) found that they competed exploitatively or via interference, depending on their ability to

take up a large volume of nectar in modified crops, and on their aggressiveness or ability to defend a flower.

*Implications for conservation management*

*Roussea simplex* is critically endangered, with less than 100 known individual plants in a few, scattered and isolated populations. Long-term survival of these populations is likely to depend on a sufficient level of pollination and seed dispersal. Our documentation of a double-disruption of two key processes in the reproduction of *R. simplex* is of major conservation concern because *P. cepediana* is currently the only known pollinator and seed disperser of the species. On the other hand, a presence of ants on the plants may be beneficial because they can provide a service by removing herbivorous insects.

*Technomyrmex albipes* ants have been observed in the majority of remaining native habitats of Mauritius, often with locally high population densities (L. Lach, pers. comm., pers. obs.). In a recent flower-visitation network study in Pétrin CMA and an adjacent heavily invaded and degraded heath area, *T. albipes* was reported visiting the flowers of 61% (45 of 74) of the native and endemic plant species in the weeded CMA, versus 41% (24 of 54) of native and endemic plant species in the invaded and degraded area (Kaiser 2006). This report supports the perhaps counter-intuitive suggestion that habitat restoration efforts in Mauritius may actually increase the impact of some groups of invasive species, exemplified by ants in this case (Ward 1990; Fisher 2005; Lach 2005). There is no doubt that many native and endemic Mauritian taxa benefit from current habitat restoration efforts. For example, successful regeneration of many endangered plants is currently restricted to the weeded CMAs (Chapter 5; Mauritian Wildlife Foundation unpublished database), native butterflies are more abundant in restored forests than in invaded forests (Mauremootoo *et al.* in press), and some endangered endemic birds preferentially hold territories within the CMAs (Edmunds 2005; pers. obs.). However, a heavily invaded native forest could still provide a good habitat for e.g. native ants that may offer biotic resistance to invasive ants (Majer 1994; Hoffmann *et al.* 1999; but see Holway 1998; Menke & Holway 2006) – until removal of all invasive plants in a single heavy weeding event creates a massive disturbance that opens the habitat for incursions by invasive ants. It is therefore crucial that future restoration efforts in Mauritius address this possible disparity in the effects of weeding on different groups of native and invasive taxa and their interactions, and modify weeding practices to benefit as large a spectrum of native and endemic taxa as possible.

Mutualistic pollination interactions of other Mauritian plants may be impacted by *T. albipes* as well. In Pétrin and elsewhere in Mauritius, we have observed *T. albipes* foraging for nectar at the flowers of several native and endemic plant species, especially vertebrate-pollinated plants with plenty of nectar; e.g. *Syzygium commersonii*, *S. mamillatum*, *S. mauritianum*, *S. petrinense*, *S. venosum* (Myrtaceae), *Labourdonnaisia callophylloides*, *Sideroxylon puberulum*, *S. cinereum* (Sapotaceae), and *Turraea rigida* and *Turrea* sp. (Meliaceae). Like at *R. simplex*, *T. albipes* builds small galleries across the corolla of several of the large-flowered of these species (*S. mauritianum, S. venosum*, and *S. commersonii*), even though their flowers only last for 1–3 days. Interestingly, we have very rarely observed *T. albipes* ants at flowers of the endemic plant *Trochetia blackburniana* (Malvaceae), even though the flowers are large, last several days, and produce great amounts of nectar (Chapter 3). However, the nectar of *T. blackburniana* is not clear like in most plants, but is rather a striking yellow colour. This species is one of the relatively few plants worldwide that produce coloured nectar (Chapter 1). In South Africa, the phenolics causing the dark brown colouration of the nectar of an *Aloe* species also render the nectar unpalatable to nectar-thieving insects (Johnson *et al.* in press). The colour pigment in *Trochetia* nectar – most likely an aurone (Olesen *et al.* 1998) – serves as a visual signal for floral reward (Chapter 2), but could have an ant-repellent function as well.

As mentioned above, *T. albipes* is widespread in mainland Mauritius; we have not found a single habitat type where the species does not occur. It is crucial, though, to prevent *T. albipes* from reaching offshore islands that have not yet been infested, such as Round Island, a small island 22 km north of Mauritius. Here, several lizard species, in an endemic reptile-dominated ecosystem, partly depend on nectar resources during some months of the year (unpubl. data; N. Zuël, pers. comm.). An accidental introduction of *T. albipes* to Round Island could have severe negative impacts on this unique ecosystem.

In conclusion, while it was realised already in 1990 that invasive ants posed a threat to native biodiversity in Mauritius (Ward 1990), the impact of invasive ants on native ecosystems in Mauritius has only recently received focused attention (Lach & Suarez 2005; 2006; Fisher 2005; this study). These studies highlight at least three different ways, in which invasive ants can negatively impact native biodiversity in Mauritius: Firstly, by incursions into newly weeded and thus heavily disturbed habitat (Ward 1990; Lach & Suarez 2005; Fisher 2005); secondly, by facilitating growth of invasive plants and spreading introduced honeydew-producing herbivores, thereby creating an ‘invasional

meltdown' (Simberloff & Von Holle 1999; Lach & Suarez 2006); and thirdly, by disrupting mutualistic interactions between native and endemic plants and animals (this study). Hence, there are several major conservation issues that need addressing in the near future: 1) the extent to which invasive ants, such as *T. albipes*, disrupt native mutualistic interactions, 2) how current habitat restoration efforts can be modified to minimise subsequent incursions by invasive ants into recently weeded areas, 3) maintaining strict quarantine regulations for offshore islands that are not yet invaded by *T. albipes* and other species of invasive ants, and 4) the feasibility of controlling invasive ants in entire habitats, or around specific, endangered target plants. The results of our study, and the above recommendations for future research and conservation management, may be applicable to other oceanic islands that face similar problems with *T. albipes* and other species of invasive ants.

## ACKNOWLEDGEMENTS

We would like to thank the National Parks and Conservation Service of Mauritius for support and permission to work in the National Park, and the Mauritian Wildlife Foundation for support. Thank you to Lori Lach for sharing unpublished data. The manuscript benefited much from a critical reading by Nancy Bunbury. The project was funded by the Swiss National Science Foundation (grant no. 631-065950 to CBM).

## REFERENCES

BAWA, K.S. (1990) Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399-422.

BLANCAFORT, X. & GOMEZ, C. (2005) Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae). *Acta Oecologica* 28: 49-55.

BOND, W. & SLINGSBY, P. (1984) Collapse of an ant-plant mutualism: the Argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. *Ecology* 65: 1031-1037.

CARNEY, S.E., BYERLEY, M.B. & HOLWAY, D.A. (2003) Invasive Argentine ants (*Linepithema humile*) do not replace native ants as seed dispersers of *Dendromecon rigida* (Papaveraceae) in California, USA. *Oecologia* 135: 576-582.

CHRISTIAN, C.E. (2001) Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature* 413: 635-639.

DONISTHORPE, H. (1946) On the ants of Mauritius. *Annals and Magazine of Natural History* 13: 25-35.

EDMUNDS, K. (2005) Resource utilisation: territory distribution and supplementary food use by the endangered Pink Pigeon *Columba mayeri* (M.Sc.). Norwich: University of East Anglia.

FEARE, C. (1999) Birds take over from rats on Bird Island, Seychelles. *Bird Conservation International* 9: 95-96.

FISHER, B.L. (2005) A new species of *Discothyrea* Roger from Mauritius and a new species of *Proceratium* Roger from Madagascar (Hymenoptera: Formicidae). *Proceedings of the California Academy of Sciences* 56: 657-667.

GODÍNEZ-ÁLVAREZ, H. (2004) Pollination and seed dispersal by lizards: a review. *Revista Chilena de Historia Natural* 77: 569-577.

GOMEZ, C. & OLIVERAS, J. (2003) Can the Argentine ant (*Linepithema humile* Mayr) replace native ants in myrmecochory? *Acta Oecologica* 24: 47-53.

HANSEN, D.M. (2005) Pollination of the enigmatic Mauritian endemic *Roussea simplex* (Rousseaceae): Birds or geckos? *Ecotropica* 11: 69-72.

HANSEN, D.M., BEER, K. & MÜLLER, C.B. (2006) Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. *Biology Letters* 2: 165-168.

HANSEN, D.M., OLESEN, J.M. & JONES, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography* 29: 721-734.

HEENAN, P.B. (1999) Extrafloral nectaries in *Elingamita johnsonii* (Myrsinaceae). *New Zealand Journal of Botany* 37: 183-185.

HOFFMANN, B.D., ANDERSEN, A.N. & HILL, G.J.E. (1999) Impact of an introduced ant on native rain forest invertebrates: Pheidole megacephala in monsoonal Australia. *Oecologia* 120: 595-604.

HOLWAY, D.A. & CASE, T.J. (2000) Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. *Animal Behaviour* 59: 433-441.

HOLWAY, D.A. (1998) Factors governing rate of invasion: a natural experiment using Argentine ants. *Oecologia* 115: 206-212.

HOLWAY, D.A., LACH, L., SUAREZ, A.V., TSUTSUI, N.D. & CASE, T.J. (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33: 181-233.

HOWE, H.F. & SMALLWOOD, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201-228.

JOHNSON, S.D., HARGREAVES, A.L. & BROWN, M. (in press) Dark bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*.

JOURDAN, H., SADLIER, R.A. & BAUER, A.M. (2001) Little fire ant invasion (*Wasmannia auropunctata*) as a threat to New Caledonian lizards: Evidences from a sclerophyll forest (Hymenoptera : Formicidae). *Sociobiology* 38: 283-301.

KOPTUR, S. & TRUONG, N. (1998) Facultative ant-plant interactions: nectar sugar preferences of introduced pest ant species in south Florida. *Biotropica* 30: 179-189.

LACH, L. (2003) Invasive ants: unwanted partners in ant-plant interactions? *Annals of the Missouri Botanical Garden* 90: 91-108.

LACH, L. (2005) Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Sociaux* 52: 257-262.

LACH, L. & SUAREZ, A.V. (2005) One restoration effort does not beget another: native ants in Mauritius do not benefit from plant restoration efforts. Abstract, XIX Annual Meeting of the Society for Conservation Biology. Brasilia.

LACH, L. & SUAREZ, A. (2006) Interactions between invasive ants and invasive plants: a potential pathway for 'invasional meltdown'. Abstract, International Union for the Study of Social Insects (IUSSI), Washington DC.

MAJER, J.D. (1994) Spread of Argentine ants (*Linepithema humile*) with special reference to Western Australia. In: *Exotic ants: biology, impact, and control of introduced species*. pp. 163-173 (Williams, D.F., ed). Westview, Boulder, CO.

MAUREMOOTOO, J.R., FOWLER, S.V., FLORENS, F.B.V. & WINDER, L. (in press) The effect of weeding alien plants on the abundance of adult butteflies in Mauritian lower montane forest. *African Entomology*.

MEEK, P.D. (2000) The decline and current status of the Christimas Island shrew *Crocidura attenuata thrichura* on Christmas Island, Indian Ocean. *Australian Mammalogy* 22: 43-49.

MENKE, S.B. & HOLWAY, D.A. (2006) Abiotic factors control invasion by Argentine ants at the community scale. *Journal of Animal Ecology* 75: 368-376.

NESS, J.H. (2003) Contrasting exotic *Solenopsis invicta* and native *Forelius pruinosus* ants as mutualists with *Catalpa bignonioides*, a native plant. *Ecological Entomology* 28: 247-251.

NESS, J.H. (2004) Forest edges and fire ants alter the seed shadow of an ant-dispersed plant. *Oecologia* 138: 448-454.

NESS, J.H. & BRONSTEIN, J.H. (2004) The effects of invasive ants on prospective ant mutualists. *Biological Invasions* 6: 445-461.

NESS, J.H., BRONSTEIN, J.L., ANDERSEN, A.N. & HOLLAND, J.N. (2004) Ant body size predicts dispersal distance of ant-adapted seeds: Implications of small-ant invasions. *Ecology* 85: 1244-1250.

OLESEN, J.M., RØNSTED, N., TOLDERLUND, U., CORNETT, C., MØLGAARD, P., MADSEN, J., JONES, C.G. & OLSEN, C.E. (1998) Mauritian red nectar remains a mystery. *Nature* 393: 529.

OLESEN, J.M. & VALIDO, A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution* 18: 177-181.

OLIVERAS, J., BAS, J.M. & GOMEZ, C. (2005) Long-term consequences of the alteration of the seed dispersal process of Euphorbia characias due to the Argentine ant invasion. *Ecography* 28: 662-672.

R DEVELOPMENT CORE TEAM (2006) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

SIMBERLOFF, D. & VON HOLLE, B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21-32.

TRAVESET, A. & RICHARDSON, D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution* Online Early.

TSUJI, K. & YAMAUCHI, K. (1994) Colony level sex allocation in a polygynous and polydomous ant. *Behavioral Ecology and Sociobiology* 34: 157-167.

VALIDO, A. & OLESEN, J.M. (In press) Importance of lizards as frugivores and seed dispersers. In: *Seed dispersal: theory and its application in a changing world* (Dennis, A., Green, R., Schupp, E., Westcott, D., eds): CABI Publishing.

WARD, P.S. (1990) The endangered ants of Mauritius: doomed like the Dodo? *Notes from the underground* 4: 3-5.

WARNER, J. & SCHEFFRAHN, R.H. (2005) Laboratory evaluation of baits, residual insecticides, and an ultrasonic device for control of white-footed ants, *Technomyrmex albipes* (Hymenoptera : Formicidae). *Sociobiology* 45: 317-330.

WARNER, J.R. (2003) Bait preferences and toxicity of insecticides to white-footed ants *Technomyrmex albipes* (Hymenoptera: Formicidae) (M.Sc.). Gainesville: University of Florida.

ZETTLER, J.A., SPIRA, T.P. & ALLEN, C.R. (2001) Ant-seed mutualisms: can red imported fire ants sour the relationship? *Biological Conservation* 101: 249-253.

# CHAPTER 8

**Exotic pest insects: another perspective on coffee and conservation**

“As soon as coffee is in your stomach, there is a general commotion. Ideas quick-march into motion like battalions of a grand army to its legendary fighting ground, and the battle rages. Memories charge in, bright flags on high; the cavalry of metaphor deploys with a magnificent gallop; the artillery of logic rushes up with clattering wagons and cartridges; on imagination's orders, sharpshooters sight and fire; forms and shapes and characters rear up. . . similes arise, the paper is covered. Coffee is your ally and writing ceases to be a struggle.”

– Honoré de Balzac (1799-1859)

"If this is coffee, please bring me some tea; if this is tea, please bring me some coffee."

– Abraham Lincoln

## ABSTRACT

Research on crop systems and biodiversity conservation in the tropics has mainly been concerned with how mid- to low-intensity agricultural systems can benefit from adjacent natural habitats by receiving ecosystem services from natural biodiversity. One intensively studied crop in this framework is coffee. Here, positive effects are relatively easy to quantify by comparing coffee yield and by recording native species diversity. However, a largely overlooked issue in the present debate is how agricultural areas affect native organisms in adjacent natural habitats, for example through movement of pest species that could impose a risk of degrading these habitats. We give an example from Mauritius, where an introduced coffee pest severely reduces the reproductive success of an endangered endemic plant species. We argue that such effects may be more common than suggested by the literature, especially when crop and native plants are congeneric. In the long term, such negative effects may degrade the natural habitats, thereby causing ecosystem services derived from these habitats to decline.

Studies in biodiversity research and conservation biology have emphasised the loss not only of species but also of ecosystem functions and resulting ecosystem services (e.g., Daily, 1997). Pollination and pest control are two examples of crucial ecosystem functions and their loss may have profound ecological, economical and social consequences (Chapin *et al.*, 2000). Animal pollination represents a critically important group of ecosystem functions, which is of particular value in agricultural landscapes (Nabhan & Buchmann, 1997; Roubik, 2002). For example, it is estimated that crop pollination by animals is worth $112 billion per year on average (Costanza *et al.*, 1997), and the decline of managed and wild pollinators is therefore a concerning trend (Allen-Wardell *et al.*, 1998; but see Ghazoul, 2005). Recent research has highlighted the role of natural habitats in maintaining a high pollinator diversity that provides stable, high levels of pollination services to nearby crop plants (Roubik, 2002; Klein *et al.*, 2003; de Marco & Coelho, 2004; Ricketts, 2004). Similarly, the natural service provided by predatory and parasitic organisms in controlling pest species on crop plants may depend on the diversity of natural habitats, in which these organisms can persist throughout their life cycles when pest insects are not available (Naylor & Ehrlich, 1997). Thus, current consensus is that the management of agricultural landscapes in the tropics should aim to maximise the benefits derived from ecosystem services rendered by animals, by maintaining structurally diverse habitats, which harbour stable populations of beneficent animal species.

One well-studied crop plant in the tropics is coffee. In many tropical montane regions, forest fragments are embedded in a matrix of traditional coffee plantations (Perfecto *et al.*, 1996; Perfecto & Vandermeer, 2002). Planting coffee bushes in proximity to forest fragments or even directly in the forest increases coffee yield because the structurally more complex habitat of the forest supports a higher diversity and abundance of pollinators and natural pest control agents for the coffee plants than agricultural, impoverished land (Moguel & Toledo, 1999; Klein *et al.*, 2003; Ricketts, 2004; Steffan-Dewenter *et al.*, 2006).

While the benefits of native animals to crop plants in the tropics are increasingly being assessed and used to inform agricultural and related conservation policies, fewer studies are concerned with the reverse impacts from agricultural practices and introduced animals on native plants in their natural habitats. The most obvious explanation for this disparity is that quantifying positive effects of e.g., pollinator diversity, or negative effects due to pest species on crop yield, is more straightforward and economically rewarding than measuring gains or losses in biodiversity in the surrounding natural habitats (Edwards

& Abivardi, 1998). While these effects on crop yield can be expressed directly in economic terms, it is more difficult to assign a universally understandable economic value to a change in natural ecosystem functioning, which can only be assessed indirectly following a decrease of biodiversity in natural habitats (Pearce, 2001).

One potential negative consequence of mixing crop plants with natural habitats could be the invasion of pest species from agricultural landscapes to the surrounding, embedded natural habitats. Certainly, the global distribution of many crop species provides a large base for invasion of pest species from agricultural landscapes to surrounding natural habitats (see Mack *et al.*, 2000). Wild hosts can provide an opportunity for pest species to build up or maintain reservoir populations before dispersing to cultivated hosts (Panizzi, 1997; Sudbrink *et al.*, 1998; Fox & Dosdall, 2003), but the role of wild hosts in pest population dynamics is usually only considered when there is an economic impact on crop yield (van Emden, 1981). Although such research bias is inevitable, it is vital to also consider the opposite view that crop plants can serve as hosts from which pests may spread into natural habitats.

Here, we add another perspective to the present debate on coffee and conservation in the tropics by presenting an example from the island of Mauritius, where an introduced coffee pest species wreaks havoc on the reproductive success of an endangered endemic plant. In Mauritius, commercial coffee *Coffea arabica* L. (Rubiaceae) plantations were established in 1721 (Rouillard & Guého, 1999). The coffee berry moth *Prophantis smaragdina* (Lepidoptera; Crambidae) was accidentally introduced to Mauritius and was first documented in 1938 (Vinson, 1938). It has long been recorded on *C. arabica* in other countries, for example on the island of Sao Tomé where it destroyed up to 80% of the coffee yield (Derron, 1977). The last reported infestation of *P. smaragdina* on coffee in Mauritius was in 1995 on plantations close to the Black River Gorges National Park, which contains the largest remaining area of native forest on the island. Preliminary observations in the National Park during another experimental study (Kaiser, 2006) suggested a strong negative effect of herbivory by *P. smaragdina* on the fruit production of the endemic dioecious shrub *Bertiera zaluzania* (Rubiaceae), which is closely related to *Coffea* (Davis *et al*., 2006). To substantiate these observations, we monitored the fruit development of 20 randomly chosen female *B. zaluzania* plants, which constitutes approximately 10% of the largest extant population on Plaine Champagne, an upland heath area within the National Park. We surveyed 10 randomly selected infructescences per plant (mean number of infructescences per plant was 21.5 ± 2.3 SE) in the first week

of February 2004 and 2005, once their fruits had started to develop and had reached a size of approximately 4 mm in diameter. In 2004, 14 out of 19 plants (flowers of one out of the 20 study plants were attacked by fungi and did not set any fruit) were attacked by *P. smaragdina* caterpillars (Figure 1a), affecting an average of 23.0% (SD ± 19.6) of infructescences in attacked plants. Within two weeks, all fruits on attacked infructescences were destroyed (Figure 1b).


**FIGURE 1.** Fruit stands of *Bertiera zaluzania* (Rubiaceae), (A) freshly attacked and (B) fully destroyed by *Prophantis smaragdina* (Lepidoptera; Crambidae). Once the developing fruits showed signs of attack, all fruits of a fruit stand were destroyed after two weeks.

In 2005, all 20 experimental plants were attacked, at a mean rate 81.3% (SD ± 21.2) infructescences per plant. This represented an increase in individual attack rate from 73.7% to 100%, and a three-fold increase in attack rate of infructescences per affected plant, compared to 2004. It is unlikely that *B. zaluzania* is the only endemic Mauritian Rubiaceae affected by this pest species, but no surveys have been carried out for any other species in the family. As in many tropical countries, the Rubiaceae is species-rich in Mauritius, where 15 genera and 59 native species occur, 88% of which are endemic to the island. Twenty-nine of these species are listed as endangered or critically endangered according to IUCN criteria (Mauritian Wildlife Foundation, unpublished database).

*Prophantis smaragdina* may become a direct threat to the reproduction of many endemic relatives of *C. arabica*, in particular the endangered congeneric *C. macrocarpa, C. mauritiana* and *C. myrtifolia*. Given that the National Park is surrounded by crops and exotic forest plantations, it is likely that associated pest species will utilise new host species among native plants in the vicinity. This may pose an additional significant threat to the critically endangered Mauritian flora and further research on this issue is needed.

Our observations from Mauritius are applicable elsewhere. In North Queensland, Australia, Blanche et al. (2002) compiled information on 49 economically important arthropod pest species, of which 31 (63%) were introduced. Nine of these species used native rainforest host plant species for at least part of their life cycle, and the author emphasized that planting crops close to the forest might not be wise.

In conclusion, we highlight the potential importance of a neglected area of agro-environmental research. It is ironic that, although these schemes are intended to both benefit from and protect areas of native habitat, they may in fact accelerate the impoverishment of such areas, and thereby ultimately compromise their own existence. Studies into such contrary effects are urgently required to counteract the largely one-sided economical approach which has dominated this emerging and active field of research to date.

## ACKNOWLEDGMENTS

We thank NPCS Mauritius for the permission to work in the National Park, MWF for logistical support, S. Ganeshan for species identification and valuable advice, and T. Good, N. Bunbury, J. Krauss, and N. M. Waser for helpful comments on earlier drafts. The project was funded by the Swiss National Science Foundation (631-065950 to CBM) and the Roche Research Foundation.

## REFERENCES

ALLEN-WARDELL, G., BERNHARDT, P., BITNER, R., BURQUEZ, A., BUCHMANN, S., CANE, J., COX, P.A., DALTON, V., FEINSINGER, P., INGRAM, M., INOUYE, D., JONES, C.E., KENNEDY, K., KEVAN, P., KOOPOWITZ, H., MEDELLIN, R., MEDELLIN-MORALES, S., NABHAN, G.P., PAVLIK, B., TEPEDINO, V., TORCHIO, P. & WALKER, S. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12: 8-17.

BLANCHE, R., BAUER, R., CUNNINGHAM, S. & FLOYD, R. (2002) Services and dis-services of rainforest insects to crops in North Queensland. Cooperative Research Centre of Tropical Rainforest Ecology and Management, Cairns.

CHAPIN, F.S., ZAVALETA, E.S., EVINER, V.T., NAYLOR, R.L., VITOUSEK, P.M., REYNOLDS, H.L., HOOPER, D.U., LAVOREL, S., SALA, O.E., HOBBIE, S.E., MACK, M.C. & DIAZ, S. (2000) Consequences of changing biodiversity. *Nature* 405: 234-242.

COSTANZA, R., D'ARGE, R., DEGROOT, R., FARBER, S., GRASSO, M., HANNON, B., LIMBURG, K., NAEEM, S., O'NEILL, R.V., PARUELO, J., RASKIN, R.G., SUTTON, P. & VAN DEN BELT, M. (1997) The value of the world's ecosystem services and natural capital. *Nature* 387: 253-260.

DAILY, G.C. (ed.) (1997) *Nature's services: societal dependence on natural ecosystems.* Island Press, Washington D.C.

DAVIS, A.P., GOVAERTS, R., BRIDSON, D.M. & STOFFELEN, P. (2006) An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society* 152: 465-512.

DE MARCO, P. & COELHO, F.M. (2004) Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* 13: 1245-1255.

DERRON, M. (1977) *Prophantis smaragdina* Butler and *Cryptophlebia colivora* Meyrick (Lepidoptera): two important pests on *Coffea arabica* L. on the island of Sao Tomé. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 50: 149-151.

EDWARDS, P.J. & ABIVARDI, C. (1998) The value of biodiversity: Where ecology and economy blend. *Biological Conservation* 83: 239.

FOX, A.S. & DOSDALL, L.M. (2003) Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. *Journal of Entomological Science* 38: 533-544.

GHAZOUL, J. (2005) Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology & Evolution* 20: 367-373.

KAISER, C.N. (2006) *Functional integrity of plant-pollinator communities in restored habitats in Mauritius*. PhD thesis. University of Zurich, Zürich, Switzerland

KLEIN, A.M., STEFFAN-DEWENTER, I. & TSCHARNTKE, T. (2003) Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* 40: 837-845.

MACK, R.N., SIMBERLOFF, D., LONSDALE, W.M., EVANS, H., CLOUT, M. & BAZZAZ, F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

MOGUEL, P. & TOLEDO, V.M. (1999) Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* 13: 11-21.

NABHAN, G.P. & BUCHMANN, S. (1997) Services provided by pollinators. In: *Nature's services: Societal dependence on natural ecosystems*, DAILY, G.C. (ed), pp 133-150. Island Press, Washington, D.C.

NAYLOR, R.L. & EHRLICH, P.R. (1997) Natural pest control services and agriculture. In: *Nature's services: societal dependence on natural ecosystems*, DAILY G.C. (ed), pp 151-174. Island Press, Washington, D. C.

PANIZZI, A.R. (1997) Wild hosts of pentatomids: Ecological significance and role in their pest status on crops. *Annual Review of Entomology* 42: 99-122.

PEARCE, D.W. (2001) The economic value of forest ecosystems. *Ecosystem Health* 7: 284-296.

PERFECTO, I., RICE, R.A., GREENBERG, R. & VANDERVOORT, M.E. (1996) Shade coffee: A disappearing refuge for biodiversity. *Bioscience* 46: 598-608.

PERFECTO, I. & VANDERMEER, J. (2002) Quality of agroecological matrix in a tropical montane landscape: Ants in coffee plantations in southern Mexico. *Conservation Biology* 16: 174-182.

RICKETTS, T.H. (2004) Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* 18: 1262-1271.

ROUBIK, D.W. (2002) Tropical agriculture - The value of bees to the coffee harvest. *Nature* 417: 708-708.

ROUILLARD, G. & GUÉHO, J. (1999) Les plantes et leur histoire à l'Ile Maurice. MSM Limited, Mauritius.

SUDBRINK, D.L., MACK, T.P. & ZEHNDER, G.W. (1998) Alternate host plants of cowpea curculio, (Coleoptera: Curculionidae) in Alabama. *Florida Entomologist* 81: 373-383.

VAN EMDEN, H.F. (1981) Wild plants in the ecology of insect pests. In: *Pests, pathogens and vegetation*, Thresh J.M. (ed), pp 251-261. Pittman Books Ltd., London.

VINSON, J. (1938) Catalogue of the Lepidoptera of the Mascarene Islands. *Mauritius Institute Bulletin* 1: 1-69.

## SUMMARY

## ZUSAMMENFASSUNG

## ACKNOWLEDGEMENTS

## CURRICULUM VITAE

"Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning".

– Winston Churchill, 1942

# SUMMARY

In my thesis I studied aspects of ecology, evolution, and conservation of plant-animal interactions on islands. My main study site was the island of Mauritius in the Indian Ocean. Mauritius is a biodiversity hotspot, with many endemic plant and animal species and high rates of extinction.

**Chapter 1** reviewed the global distribution, ecology and evolution of coloured nectar, a rare floral trait that is particularly widespread on islands (including Mauritius) and insular mainland habitats such as mountains. Once thought to be restricted to three endemic plants in Mauritius, we showed that this is not the case: coloured nectar is found in more than 60 species from many plant families around the world. We also discussed the evolution of coloured nectar, and speculated on its ecological function.

In **Chapter 2**, we experimentally tested a hypothesis from Chapter 1 about the possible ecological function of coloured nectar as a signal for floral reward. We used endemic flower-visiting geckos in Mauritius as our study organism, and found strong support for the signal-hypothesis, with geckos strongly preferring coloured over clear nectar. Thus, the chapter – at least partly – solved the mystery of the Mauritian coloured nectar.

**Chapter 3** demonstrated that the endemic Mauritian plant *Trochetia blackburniana* (Malvaceae) is pollinated by the endemic *Phelsuma cepediana* gecko, but that this interaction is structured by the indirect effects of proximity to patches of *Pandanus* (Pandanaceae) plants – a favoured microhabitat of the geckos. Proximity to *Pandanus* patches lead to higher gecko visitation rates and a subsequently higher fruitset in *T. blackburniana*. Some studies have shown how two or more flowering plant species can positively or negatively affect each other's reproductive success through indirect effects mediated by shared pollinators. In contrast, Chapter 3 gives a unique example of a non-flowering plant affecting the reproductive success of a neighbouring flowering plant.

**Chapters 4 and 5** together formed a detailed study of the pollination and the seed dispersal ecology of the critically endangered endemic Mauritian tree *Syzygium mamillatum* (Myrtaceae). **Chapter 4** showed how weeding of invasive plant species can influence the reproductive success of *S. mamillatum* in the weeded habitat, based on differences in pollinator behaviour between weeded and unweeded sites. In **Chapter 5**, we provided the first experimental evidence of the importance of the Janzen-Connell model

for seedling establishment on oceanic islands, and demonstrated how ecological analogue species can be used to resurrect extinct seed dispersal interactions.

**Chapters 6 and 7** comprised a study of the pollination and seed dispersal interactions of another critically endangered Mauritian endemic plant, *Roussea simplex* (Rousseaceae), and how an invasive ant affects both interactions detrimentally. **Chapter 6** showed that endemic *Phelsuma cepediana* geckos currently are the sole pollinators and seed dispersers of *R. simplex*, and **Chapter 7** experimentally demonstrated that a presence of the invasive ant *Technomyrmex albipes* at *R. simplex* flowers or fruits scare away the geckos, thus rendering the plant without pollinators and seed dispersers.

In **Chapter 8** we documented the strong negative effects of a coffee pest species on the reproductive success of the endangered Mauritian endemic plant *Bertiera zaluzania* (Rubiaceae). Our study highlighted another perspective to the ongoing scientific debate about coffee as a cash crop and the maintenance of biodiversity in the tropics. Most current studies focus on the benefits that coffee plants can derive from nearby natural habitats, and neglect to investigate the potential detrimental effects of coffee pest species invading these natural habitats.

# ZUSAMMENFASSUNG

In dieser Dissertation präsentiere ich Studien, die sich mit der Ökologie und der Evolution, als auch mit Aspekte des Naturschutzes und der Erhaltung der biologischen Vielfalt von Tier-Pflanze-Interaktionen auf ozeanischen Inseln befassen. Mein Hauptuntersuchungsstandort ist die Insel Mauritius im Indischen Ozean. Mauritius besitzt einen ausgeprägten Endemismus und eine hohe Aussterberate und wird daher als 'Biodiversitäts-Hotspot' bezeichnet.

**Kapitel 1** umfasst ein Review über die globale Verbreitung farbigen Nektars, eines seltenen Blütenmerkmals, das besonders häufig in Pflanzen auf Inseln (u.a. auf Mauritius) und in insulären Habitaten (wie z. B. Bergspitzen) anzutreffen ist. Wir zeigten, dass dieses Blütenmerkmal in mehr als 60 Arten aus vielen verschiedenen Familien rund um den Globus vertreten ist. Wir diskutierten die Evolution farbigen Nektars und spekulierten über mögliche ökologische Funktionen.

In **Kapitel 2** untersuchten wir mit Hilfe eines Experiments eine aus Kapitel 1 abgeleitete Hypothese: farbiger Nektar dient als ein Signal für das Vorhandensein von Blütenprodukten. Um diese Hypothese zu testen, offerierten wir endemischen Taggeckos in Mauritius gefärbten und klaren Nektar. Die Wahl fast ausschliesslich gefärbten Nektars unterstützt die Signal-Hypothese. Unsere Veröffentlichung trägt dazu bei, das Geheimnis um mauritischen farbigen Nektar zu lüften.

Wir beweisen in **Kapitel 3**, dass die endemische Pflanzenart *Trochetia blackburniana* (Malvaceae) von der endemischen Taggeckoart *Phelsuma cepediana* bestäubt wird. Diese Tier-Pflanze-Interaktion wird jedoch durch indirekte Effekte beinflusst, die im Zusammenhang mit den benachbarten Pflanzenarten der Gattung *Panadanus* (Schraubenbaum; Pandanaceae) stehen. Pandanusarten gehören zu den geeigneten Lebensräumen der Geckos, und *T. blackburniana* konnte höhere Besuchsraten und einen höheren Fruchtansatz verzeichnen, wenn es in der Nähe von *Pandanus* wuchs. Einige Studien konnten zeigen, dass der Fortpflanzungserfolg zweier benachbarte Pflanzenarten indirekt, durch gemeinsame Bestäuber, von der Anwesenheit der zweiten Art beeinflusst werden kann. Im Gegensatz dazu zeigten wir in Kaiptel 3 das einmalige Beispiel, dass eine nicht-blühende Pflanze eine blühende Pflanze indirekt beeinflusst.

**Kapitel 4 und 5** umfassten detaillierte Studien über die Bestäubungsbiologie und Mechanismen der Samenverbreitung der stark gefärdeten endemischen Baumart *Syzygium*

*mamillatum* (Myrtaceae). **Kapitel 4** beschreibte, wie sich das Entfernen von eingeführten, invasiven Pflanzenarten auf den Fortpflanzungserfolg von *S. mamillatum* in restaurierten Gebieten auswirkt, ein Unterschied, der auf das Verhalten von Vögeln als Bestäuber zurückgeführt werden kann. In **Kapitel 5** lieferten wir den ersten experimentellen Beweis für die Wichtigkeit des Janzen-Connell Modells über die Etablierung von Keimlingen auf einer ozeanischen Insel. Zudem demonstrierten wir, dass analoge Arten, die in ihrer ökologischen Funktion den ehemaligen, jetzt ausgestorbenen Arten nahestehen, als geeignete Samenverbreiter von *S. mamillatum* in Frage kommen.

Die **Kapitel 6 und 7** beschrieben Bestäubung und Samenverbreitung der stark gefährdeten, endemischen Pflanzenart *Roussea simplex* (Rousseaceae), und wie eine eingeführte Ameisenart negative Folgen auf die Reproduktion dieser Pflanze haben kann. In **Kapitel 6** zeigten wir, dass der endemische Taggecko *Phelsuma cepediana* der einzige Bestäuber und Samenverbreiter von *R. simplex* ist. **Kapitel 7** hingegen beweist experimentell, dass die Präsenz der invasiven Ameisenart *Technomyrmex albipes* auf Blüten und Früchten von *R. simplex* Taggeckos verscheucht, was zu einer Reduktion der Bestäubung und Samenverbreitung bei *R. simplex* geführt hat.

In **Kapitel 8** dokumentieren wir einen erheblichen, negativen Einfluss eines Kaffeeschädlings auf den Fortpflanzungserfolg der gefährdeten mauritischen Pflanzenart *Bertiera zaluzania* (Rubiaceae). Unsere Arbeit trägt eine weitere Perspektive zu der anhaltenden, wissenschaftlen Debatte bei, die sich mit dem Einfluss von Kaffeeplantagen auf die Erhaltung der biologischer Vielfalt in den Tropen beschäftigt. Die meisten Studien befassen sich mit den Vorteilen des Kaffeeanbaus in der unmittelbaren Umgebung von natürlichen Habitaten, wenige Arbeiten jedoch untersuchen die nachteiligen Effekte, die eingeführte Kaffeeschädlinge auf den benachbarten Lebensraum ausüben können

.

# ACKNOWLEDGEMENTS

First of all, I want to express my gratitude to my supervisor, Christine Müller, for believing in what must have seemed like mad ramblings about how Mauritius, coloured nectar, islands, conservation, and pollination and seed dispersal could be put together in one thesis. I still can't believe it took only one bottle of red wine to convince her to supervise me. I don't think I could have had more freedom to explore nerdy avenues of research than I have had with you, Christine, and I am very grateful for that (and for you investing in a wonderful digital SLR camera for the research group, which kept my inner photo-daemon happy throughout the years). Second, I would not have managed to do all the work I did in Mauritius without my always helpful and committed colleague, friend and co-nerd, Christopher Kaiser. Ze German in ju iz ztrong (and hence I also gratefully acknowledge your German translation of my summary!), and I must admit that the far superior 'The Box' was a good investment. Countless hours together in the field, on Camp track, in Latvia (!), on Henrietta with a G'n'T – and lately rather 'a few' hours in our office in Zurich – have cemented a friendship that I hope will result in tropical inter-island exchange of visits in many years to come. I would also like to thank the members of my PhD-committee, Elena Conti, Peter Linder, and Lukas Keller, for discussions and support.

Mauritius. This island has occupied a large part of my heart and head for many years now, and I have been extremely lucky in working alongside some of the most dedicated biologists and conservation workers on this planet. I want to thank the National Parks and Conservation Service (NPCS) and the Forestry Service (FS) for invaluable help and permission to work in the forests of Mauritius, and in particular thank you to Yousouf Mungroo, Vishnu Bachraz, Mario Allet, Rafik Jumoorty, Suraj Gopal and Paul Moolee (NPCS), and to Raj Rutty and Dan Ponnusawmy (FS). None of my work in Mauritius could have been accomplished without the close collaboration with the Mauritian Wildlife Foundation (MWF) over the years. First and foremost, I want to thank Wendy Strahm and Carl Jones for believing that it was not too late to save what little remained of Mauritian biodiversity in the first place. Among all the great Mwaffers, I want in particular to thank the crew at Camp for their company, friendship, help – and for keeping me more or less sane during many months in the rain and mud: Alpha, Jason 'coffee-bro' Malham, Nancy Bunbury, Tom Bodey, Paul Freeman, Pete Haverson, Phil Jackson, Mike 'Hunt' Sharp, Jannie Linnebjerg, Anna Reuleaux, Steeves Buckland, Sam McKecknee, Gareth Kett, Maddie Charlton, Bronwyn McCulloch, Steph Freeman, Kelly Edmunds and Andy Plant.

Special thanks to our faithful 'steed-on-wheels', Latvia. She was incredibly well-equipped, and far superior in so many ways. Many other Mwaffers and researchers from other fieldstations have helped a lot during the years and become friends: Jessie, Rachel Atkinson, Jean-Claude Sevathian, Chrissy Griffiths, Shiva Sawmy, Iben Sørensen, Cristian Jensen, Nicole Lohrmann, Kathrine Kreutzer, Dave Stepnisky, Nicolas Zuël, Amanda Ladkoo, Nick Cole, Luke Harmon, Dave Wills, Andy Cristinacce, Lori Lach & Manfred; and all other Mwaffers! Last, but not least, the MWF office staff, who have always supported me in the field: Isabelle Lenoir (the true heart of the office!), Vikash Tatayah, Lone Raffray, John Mauremootoo, Yacoob Mungroo, Sidick Calloo, Nadine Armelle, Roslyn Lagesse, Debby de Chazal, and Ashok Kundun. Other people in Mauritius have also helped tremendously, and become friends: First and foremost the one and only Gabriel d'Argent, whose encyclopedic knowledge of the Mauritian flora, and whose unrivalled field skills – even above the age of 80! – never fails to inspire. Similarly, I thank Pere Wiehe for sharing his immense knowledge about plants, and for showing me how a catholic priest can combine hiking and cold beer. Vincent Florens from the University of Mauritius was always ready to share his knowledge and enthusiasm with me, as was Claudia Baider of the Mauritius Herbarium, and I thank them both. Although not much in Mauritius anymore, I want to thank Anthony Cheke for enthusiastic discussions and for being allowed to tap into his immense knowledge of all things Mascarene. During our 'tortoise-poo' experiments, it was a particular pleasure to go to La Vanille Crocodile and Tortoise park once a week, and stumble across yet another piece of the brilliant chocolate cake offered there – special thanks to Owen Griffiths and Gilbert Moutia for all their help, and for allowing us to use some of the precious tortoises. Further culinary thanks go to Cabanon Créole, and to Prem for making the best sandwiches in Mauritius. I also had the good fortune of being able to lure two of my Danish friends down to Mauritius, to join me as field assistants – and I want to thank them for all their help: Lotte 'Das Löttchen' Reiter and Heine 'Dyredh Igor/Træl' Kiesbüy, may pwunes and spiders follow you always!

Back in Zurich, it was always difficult to readjust to 'city-life' after months in the field in Mauritius ("what do you mean, I can't simply spit out tooth paste on the floor?", "mental memo: not all cats are bad..."). However, this was made much easier by the extraordinary open atmosphere at the Institute of Environmental Sciences (IfU) and the IfUnarians 'living' there. In particular, I want to thank my Müller-lab-co-PhD-students, Chris(-ee-Baip) Kaiser, Simone(-ee-Baip) Härri (who taught me that aphids and grass can

be exciting; no small feat! –and who kept me (in)sane on many occasions...), and Nicolas Zuël for being the best group of colleagues one could hope for – and I here also include the honorary group member Nance(-ee-Baip) Bunbury, who graced us with her dry English wit on many occasions (and whose eyes and brain scrutinised several of the chapters). Also, big cheers to the 'cross-over' Diploma-students, who worked both in Mauritius and Zurich: Karin Beer, Julia Nüsscheler, and Gabor von Bethlenfalvy. Further thanks to my other office mates, Chris Philipson, Nicolas Zuël, Eva Vojtech and Steffi von Felten for making it such a good time there. Cheers also to some of the people from the neighbouring office: Kiwi-Alex and his academic wife Jena-Jana, and to Sophie Hoehn for being the angel of networks & computers (and for being there for a good laugh as well). Thanks to Bernhard Schmid for creating an open, encouraging atmosphere at the IfU, and for always having time for a discussion on the deeper meaning of statistics; to Isabelle Schöchli for her never-ending help with the jungle of bureaucracy, and for her great team spirit; to Jochen Krauss for help and suggestions, and for being a loveable, grumpy guy with a great sense of humour; to Andy Hector and Lindsay Turnbull for statistical advice and long-term loan of Black Books; to Helmi Brandl for help with the HPLC analyses – and to all other IfUnarians!

During my time in Zurich, I spent some two years, on and off, as a visiting lab-rat in the molecular lab of the Institute of Systematic Botany. It was my aim to construct a molecular phylogeny of *Trochetia* and related genera, but –alas!– the Gods of PCR did not grant me my wish, hence there is sadly not one iota of that work in the present thesis. However, I interacted with some amazing people out there, and I would like to thank them for all their help and input: first of all Timo van der Niet, for being incredibly patient with an ecologist from the rainforest, for never giving up hope, and for teaching me that DNA denatures at 140°C; Chloé Galley for being wonderfully British; and Peter Linder, Elena Conti, Peter Endress, and Mary Endress for many stimulating discussions.

Somehow, I also managed to travel around a bit to various conferences and universities, where I met some truly inspiring people: Thank you to Michelle Zjhra for hosting me in the US, and for nerdy discussions about pollination, cauliflory and biogeography; to Christophe Lavergne for helping me in La Réunion; to Luc Gigord in Lausanne for being a fellow Mascarene nerd; to the rest of the coloured nectar crew, Jens Mogens Olesen, Tom Mione and Steve Johnson; and a great chunk of my heart goes to the Swiss-American-Venezuelan übernerd Tatjana Good for being who she is; I wouldn't want it any other way!

When I came to Zurich from Aarhus in Denmark, I geographically left some people behind, but they continued to give me support and friendship from afar: First and foremost, Jens Mogens Olesen, my mentor, who first turned me to the dark side of pollination biology on islands. Similarly, after our joint adventures on the Canary Islands, Yoko Dupont and Alfredo Valido have always been there for me, with help and obscure references. A special thank to the Great Dane, Jannie Linnebjerg, for her faith in my qualities as a supervisor, and for help in the field in Mauritius; and to Maria Norup for being a fellow nerd of the highest class. Three cheers for a handful of other people in Denmark, who have made me feel missed, and whom I will always look forward to visit: Søren Nissen, Peter Friis Andersen, Karen Clausager, Dorte Friis Nyhagen, David Clement and Mads Flyvholm.

The work was supported financially by a grant from the Swiss National Science Foundation (grant no. 631-065950 to Christine Müller), and I gratefully acknowledge additional financial support from Bernhard Schmid.

As already mentioned on the first page, I dedicate this thesis to my parents, for letting me run around and get muddy in the forests of my childhood.

# CURRICULUM VITAE

***PERSONAL***

| | |
|---|---|
| Surname: | Hansen |
| First names: | Dennis Marinus |
| Date of Birth: | 24-04-1972 |
| Nationality: | Danish |

***EDUCATION***

| | |
|---|---|
| Highschool: | Aabenraa Gymnasium & HF, Denmark, 1991 |
| University: | M.Sc. in Biology, University of Aarhus, Denmark, 2001 |
| M.Sc. Thesis: | "Trees, birds, and bees in Mauritius. Endemic plant–animal interactions, introduced honey bees and conservation"<br>Supervisor: Prof. Jens Mogens Olesen |
| Ph.D. Thesis: | "Ecology, evolution, and conservation of plant–animal interactions on islands"<br>Institute of Environmental Sciences, University of Zurich, Switzerland, 2003–2006.<br>Supervisor: Prof. Christine B. Müller |

***PUBLICATIONS***

* = publications resulting from M.Sc. study

** = publications resulting from Ph.D. study

*PEER-REVIEWED*

**KAISER C. N., **HANSEN D. M.** & MÜLLER, C. B. (in press) Pollination ecology of the rare endemic tree *Syzygium mamillatum* in restored and unrestored habitats in Mauritius. *Biotropica*

**KAISER, C.N., **HANSEN, D.M.** & MÜLLER, C.B. (in press) Exotic pest insects: another perspective on coffee and conservation. *Oryx*

****HANSEN, D.M.**, KIESBÜY, H.C., JONES, C.G. & MÜLLER, C.B. (2007) Positive indirect interactions between neighbouring plant species via a lizard pollinator. *American Naturalist* 169: 534–542

****HANSEN, D.M.**, OLESEN, J.M., MIONE, T., JOHNSON, S.D. & MÜLLER, C.B. (2007) Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. *Biological Reviews* 82: 83–111

****HANSEN, D.M.**, BEER, K. & MÜLLER, C.B. (2006) Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. *Biology Letters* 2: 165–168.

**HANSEN, D.M.** (2005) *Phelsuma ornata* (ornate day gecko). Tail loss. *Herpetological Review* 36: 453.

***HANSEN, D.M.** (2005) Pollination of the enigmatic Mauritian endemic *Roussea simplex* (Rousseaceae): Birds or geckos? *Ecotropica* 11: 69–72.

DUPONT, Y.L., **HANSEN, D.M.**, RASMUSSEN, J.T. & OLESEN, J.M. (2004) Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology* 18: 670–676.

DUPONT, Y.L., **HANSEN, D.M.**, VALIDO, A. & OLESEN, J.M. (2004) Impact of introduced honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation* 118: 301–311.

DUPONT, Y.L., **HANSEN, D.M.** & OLESEN, J.M. (2003) Structure of a plant-flower-visitor network in the high- altitude sub-alpine desert of Tenerife, Canary Islands. *Ecography* 26: 301–310.

***HANSEN, D.M.**, OLESEN, J.M. & JONES, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography* 29: 721–734.

VALIDO, A., DUPONT, Y.L. & **HANSEN, D.M.** (2002) Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. *Acta Oecologica* 23: 413–419.

*BOOKS AND POPULAR SCIENCE*

DUPONT, Y.L., HANSEN, D.M., VALIDO, A. & OLESEN, J.M. (2004) Introduced honeybees on islands: impacts on native pollination interactions. *Ecologia Mediterranea* 30: 104-105.

DUPONT Y.L., **HANSEN D.M**., VALIDO A. & OLESEN J.M. (2004) Introduced honeybees on islands: impacts on native pollination interactions. *Ecologia Mediterranea*, 30, 104-105

**ATKINSON. R., SEVATHIAN J.C., VIRAHSAWMY, M., AH-KING, J., CLAYTON, J., KAISER, C.N., **HANSEN, D.M.**, CRANWELL, S. AND MAUREMOOTOO, J. (2003) *A field guide to the common trees & shrubs of upland forests in Mauritius*. Mauritian Wildlife Foundation, Vacoas, Mauritius.

**HANSEN, D.M.** (2001)(in Danish) Blomster og farver – set fra bestøvernes synsvinkel. *Værn om Synet* 18(1): 3-5

*SCIENTIFIC PHOTOGRAPHY*

**ATKINSON. R. & SEVATHIAN J.C. WITH PHOTOS BY C.N. KAISER & **D.M. HANSEN** (2005) *A guide to the plants in Mauritius*. Mauritian Wildlife Foundation, Vacoas, Mauritius.188 pp.

**GOPALL, J., AH-KING, J., ATKINSON. R., SEVATHIAN J.C. WITH PHOTOS BY **D.M. HANSEN**, C.N. KAISER & OTHERS (2005) *The native plants and animals of Mauritius*. Mauritian Wildlife Foundation, Vacoas, Mauritius. 47 pp.

Dodo sketch by Roelandt Savery, 1626